Summary of Discussions from Breakout Sessions during the USDA/APHIS public meeting on Genetically Engineered Trees, July 8-9, 2003.

The breakout sessions held at this meeting were designed to elicit comments and stimulate discussion among meeting participants regarding a number of questions that the steering committee felt was important to a number of issues surrounding the possible deregulation of genetically engineered tree species. This paper has been put together as a summary of the discussions in each group and does not necessarily capture every individual comment expressed. In the course of discussions, a number of questions arose and those are summarized at the end of the 2 primary sections of the document (forest and fruit trees).

Forestry Breakout Groups:

I. Gene flow

A. Are there recommended methods to evaluate gene flow issues? What are those methods? Are there particular genes that would be more suitable than others for this work?

There is some literature currently available that would be applicable to evaluate gene flow issues. In the case of genetically engineered trees, however, this work is not possible to do without large scale field trials. Computer modeling can likely be useful in predicting amounts of gene flow (primarily pollen and seed movement by wind). It is also important to consider, however, the likelihood of asexual/clonal propagation of some species. If one considers doing this research, either DNA markers or the gene for GFP (green fluorescent protein) were suggested as tools for these studies. Studies could also be done with non-transgenic traits by measuring their introgression with related species. In general, there is a need to distinguish among gene flow, environmental persistence/ introgression and increased fitness characteristics of particular species/trait combinations.

B. Would the use of plant or seed sterility systems be useful in some cases? Which cases would those be? Are there other strategies that would also be useful to address gene flow issues?

As with many of the issues involved with genetically engineered plants, one of the continuing and recurring themes is that determinations should be evaluated on a case-by-case basis. Sterility systems might be useful or preferred if there were reason to believe that a problem would arise if not used. A couple of those times might be when growing within or close to a center of origin and where hybridization/introgression probabilities are significant. If, however, a trait has been determined to be benign (or neutral) and provide no selective advantage, then sterility might not be needed. Other methods of gene containment/management can also be useful (species barriers, self incompatibility, altered flowering response, plantation management practices, border rows, etc). Sterility development alone will not address all gene flow issues. There are some cases where sterility is not desired (e.g., blight resistant American chestnut).

> C. Assuming that genes without increased fitness characteristics may persist in the environment, should APHIS evaluate the impacts of this and if so, how should this be accomplished?

This topic garnered numerous comments that lead to more questions. In general, it seems that issues relating to increased or decreased fitness and evaluating their impact may be quite difficult to determine within a reasonable time frame. Fitness characteristics may be quite different in different environments and different locations. Even genes without increased fitness characteristics could genetically swamp small forests near large plantations. Regulations designed to minimize the likelihood of transgene escape into non-domesticated populations make evaluating potential fitness characteristics very difficult. Can it be determined, *a priori*, which genes will increase, decrease or have no effect on plant fitness in a particular species in a particular location? To some groups the primary concern is "genetic integrity" and the presence of transgenes themselves in a population are an impact. Some mention was made of a possible "Trojan gene" scenario with respect to plant fitness and whether that might be of concern.

A "Trojan gene hypothesis" was put forth by researchers at Purdue University (Muir and Howard, 2000) using computer modeling and statistical analysis. Using specific fitness and fertility characteristics of a GE salmon, the computer model predicted extinction of some natural salmon populations in as little as 20 generations. The researchers do point out, however, that both the genetic background of the organism and the release environment are critical to determining real risk.

Evaluating the current list of characteristics in Appendix II, keeping the trait and species in mind might be a good starting point.

D. Other than the following: outcrossing frequency, compatible species, known hybrids, weedy or invasive nature of compatible species, are there other parameters related to outcrossing that should be evaluated?

The only further suggestions for evaluation were fitness, genetic load and possible changes in dispersal mechanisms (i.e., wind, animal).

Genetic load has generally been defined as the decrease in fitness of a population (as a result of selection acting on phenotypes) due to deleterious mutations in the population gene pool. More specifically, the average number of recessive lethal mutations, in the heterozygous state, estimated to be present in the genome of an individual in a population.

II. Weediness/invasiveness

E. Other than seed characteristics of production, dormancy, emergence and viability, are there other seed parameters that should be evaluated?

Modeling was suggested to see if expected changes might affect invasiveness. Changed propensity for root suckering was additionally suggested for evaluation.

F. How could the spread of herbicide resistance genes into compatible species of trees of interest affect weed populations and weed management options?

In the absence of selective use of an herbicide, the presence of herbicide tolerance genes may be unimportant. In some locales where an herbicide (e.g., glyphosate) is highly favored, other control chemicals may make control a challenge. Alternate control methods and/or a stewardship plan should be developed.

III. Other Issues

G. What research tools are available to extrapolate results obtained from controlled field trials over relatively short time frames to large scale plantation and orchard plantings?

It was noted that there is over 50 years of experience in standard tree improvement data and information. Literature is available to make juvenile→ mature tree sort of correlations. Microarray technology can be used to monitor the physical state of trees to develop models with predictive capabilities. Existing models allow selection of superior phenotypes as early as 4 years while clonal systems sometimes allow selection even earlier. However, similar sorts of studies may need to be done with genetically engineered trees in order to validate similar relationships. Modeling may not, however, give good information on ecosystem impacts or identify keystone species or processes. It was also pointed out that different flora/fauna communities exist in plantings of juvenile versus mature trees. Attempting to address possible impacts in all the different ecosystems where specific trees might be grown could be quite cumbersome. Adaptive management was recommended as a tool to address many of the issues associated with large scale plantings of genetically engineered trees. The British Columbia Forest Service defines adaptive management as:

a systematic process for continually improving management policies and practices by learning from the outcomes of operational programs. It's most effective form–"active" adaptive management employs management programs that are designed to experimentally compare selected policies or practices, by evaluating alternative hypotheses about the system being managed.
http://www.for.gov.bc.ca/hfp/amhome/Amdefs.htm.

H. Are greenhouse and/or growth chamber tests sufficient for some of the recommended traits?

For some traits (e.g., pollen viability or others) this testing might be fine or could supplement field data.

I. How should APHIS evaluate the impact on ecosystems resulting from introduction of plants with pesticidal properties and the likely development of resistant pests?

To a great extent, EPA has regulatory authority and will need to weigh in heavily on the issues associated with plant-incorporated-protectants (PIP's) in trees as they have done in annual crops. Beyond that, some of these genes (particularly Bt types) are likely to be keystone genes and ecosystem evaluations may be impossible to do without large scale trials. Development of lepidopteran resistant (Bt) trees in the US has been quite slow, primarily due to limited funding and identified concerns with possible development of resistant pests. Blight resistant American chestnut was identified as a different case and separate from Bt. The issue of sustainability was discussed as it pertains to effectiveness of a product/pesticide over some defined time frame (i.e., is there a minimum required time frame that a tree should resist pests prior to the development of resistant organisms?).

J. Is the non-transgenic progenitor organism the appropriate comparator in all cases? If not, what would be the appropriate comparator?

A non-transgenic iso-line is appropriate in most cases (e.g., pine, poplar). For some species breeders have lines that are commonly used as standards for comparison. In some cases this may depend on the trait being examined and the appropriate comparator(s) should be determined based on the range of expression/variation within the species. Domestication of trees is part of a long term process and although some traits might be outside the "typical" range of expression of a species, a tree should still be considered useful if it performs well in a plantation setting.

Comments on the list of proposed characteristics outlined in Appendix II of the US/Canada bilateral agreement:

1. Stress adaptations:
    - Normal observations associated with a variety development program are sufficient for analysis.

2. Evaluation of plant hardiness:
    - The range of comments reflected the following: 1) the suggested testing in the meeting document is sufficient, 2) hardiness is not normally evaluated in a conventional program, 3) there is no a priori reason to perform this testing.

3. Effects on symbiont organisms:
    - The range of comments reflected the following: 1) these studies are probably not useful/ microflora is highly adaptable to change, 2) this testing is not normally performed, 3) based on current literature, if specific traits are found to be important they can be studied in lab/greenhouse studies.

Additional comments and questions that came out of the forest group breakout session discussions:

- Given the long time frames involved in evaluating trees, an adaptive management approach was suggested to gather data along with some sort of "conditional" deregulation.
- It appears that there is a gap in the national research system regarding funding for evaluating economic and ecosystem effects of GE trees. Agencies within USDA need to make this connection.
- Scientific panels/ public meetings are a useful means for APHIS to gather information.
- Involve scientific societies in the process for regulatory decision-making in a way that provides for some external review.
- Tracking transgene movement is important scientifically but what is important to APHIS as a regulatory agency?
- Is what we currently know about poplar and loblolly pine pollen movement sufficient for satisfying APHIS requirements for deregulation?
- Is total gene flow control the goal or is there an allowable level?
- Are gene flow restrictions needed, particularly with reduced fitness traits?
- Are gene flow studies needed for an APHIS decision? Are they desired for monitoring after deregulation?
- What is being measured to determine increased fitness?

Fruit tree breakout groups:

I. Gene flow

A. Are there recommended methods to evaluate gene flow issues? What are those methods? Are there particular genes that would be more suitable than others for this work?

Gene flow from engineered to non-engineered fruit and/or nut trees may be a concern. Although the edible "fruit" of apples, plums and others would not be transgenic, the seed certainly will be if pollen moves. Some feral plants may survive on the perimeter of orchards but normal orchard management practices eliminate seedlings within an orchard. Management may also eliminate perimeter feral plants that could serve as sources of disease or pest problems. In some cases, gene flow into wild populations may be beneficial to address diseases (e.g., plum pox virus resistance since there is no current source of resistance in the gene pool). There is significant literature on pollen movement to serve as base knowledge. One possible complicating factor is that bee hives are sometimes moved from one location to another for pollination purposes. Current field testing requirements do not, however, allow for evaluating gene flow from transgenic plots.

B. Would the use of plant or seed sterility systems be useful in some cases? Which cases would those be? Are there other strategies that would also be useful to address gene flow issues?

To a large extent, fruit trees differ significantly on this issue from forest trees in that pollination is usually required to obtain edible fruit. Issues with nut trees and the greater existence of compatible relatives are more similar to forest species. Chloroplast transformation would solve the issue of pollen movement but not seed. If there is a likelihood of increasing plant fitness, sterility might be a useful tool. It would be important to evaluate the likely impact of the gene in the wild population. The issues point to case-by-case analysis and possible “conditional” deregulation. Although the possibility of seed planting by individuals is possible, it is not highly likely and most seed grown fruit trees do not compete well.

C. Assuming that genes without increased fitness characteristics may persist in the environment, should APHIS evaluate the impacts of this and if so, how should this be accomplished?

Some determination of fitness and the ability to survive is important but data should support such a determination. If there is no increased fitness, there should be no need to evaluate. Evaluate case-by-case with particular note to presence of wild relatives. Use known data from non-transgenics and consider monitoring post deregulation.

D. Other than the following: outcrossing frequency, compatible species, known hybrids, weedy or invasive nature of compatible species, are there other parameters related to outcrossing that should be evaluated?

The role of insect and other possible pollinators should be assessed. Animal dispersal of seed can be a source of gene flow. Although movement of bees to organic farms/crops may occur, current APHIS regulations only allow plant pest risk analysis.

II. Weediness/Invasiveness

E. Other than seed characteristics of production, dormancy, emergence and viability, are there other seed parameters that should be evaluated?

Two groups talked about dispersal by birds. At least a couple items could be considered: change in palatability by birds and the possibility of a change in seed viability and seedling survival post bird consumption. A case-by-case analysis would likely be required. In the case where disease resistance genes might give a competitive advantage to hybrids with wild relatives, the hybrid feral population might not only become more fit but could serve as a source of disease inoculum or other pathogens. With a broad range of natural variability in disease and insect resistance within species, evaluation of this diversity will likely be a critical consideration.

F. How could the spread of herbicide resistance genes into compatible species of trees of interest affect weed populations and weed management options?

For the most part, there are few programs working on development of herbicide resistant fruit or nut trees. If this trait were used as a selectable marker then it could present management problems if the trait were passed to compatible wild species. Were herbicide resistance to occur (possibly in pear, apple, or brambles), herbicide resistance would reduce current management options.

III. Other Issues

G. What research tools are available to extrapolate results obtained from controlled field trials over relatively short time frames to large scale plantation and orchard plantings?

In approximately 5 years, most fruit trees are considered to be mature. This may not be the case with nut trees which, in some cases, take much longer. Current tools for extrapolation may not be well developed; however, there are over 100 years of experience with breeding and research trials from which to draw. Ecosystem effects are probably less important to evaluate compared to forest trees given the highly managed nature of an orchard setting. In the case of virus resistance and the in-planta mechanism, it may be important to assess the potential loss of effectiveness of silenced genes.

H. Are greenhouse and/or growth chamber tests sufficient for some of the recommended traits?

Greenhouse and/or growth chamber testing may be suitable for evaluating some traits but it should be on a case-by-case basis. Traits such as cold hardiness, virus resistance (and possibly other pathogen resistance) and nutrition studies may be suitable and testing more dependable. Evaluation of traits where there may be complex interactions relating to vigor, multiple insect exposure or yield would likely not be useful. In most cases these studies should further be verified by field testing where possible. Currently literature should be highly useful to determine if traits can be convincingly evaluated in a greenhouse.

I. How should APHIS evaluate the impact on ecosystems resulting from introduction of plants with pesticidal properties and the likely development of resistant pests?

Plant-incorporated-protectants (PIP’s) (genes for virus, bacterial or fungal resistance) fall under EPA regulatory authority. Two groups commented that either change in insect population resistance is unlikely to be affected or that any changes might be desirable and therefore make this issue irrelevant. If this issue were a concern in the orchard or surrounding ecosystem, normal tests for insect resistance should be applied or this could be addressed with refuges of non-PIP containing plants (as has been required by EPA of all Bt crops). Consideration should be given to benefits that could accrue from introduction of specific resistance genes to an invasive species for which there might be no other method of control (e.g., plum pox virus and chestnut blight).

J. Is the non-transgenic progenitor organism the appropriate comparator in all cases? If not, what would be the appropriate comparator?

Three primary points were made overall. The non-transgenic progenitor is most useful. Comparison with a range of related cultivars is highly useful as references to demonstrate variation within the species. Since rootstocks are used extensively in orchards, one or several appropriate rootstocks should be used in comparison testing. The point regarding rootstocks is significant in that rootstocks can have significant effects on plant growth and development compared to own-rooted trees.

Comments on the list of proposed characteristics outlined in Appendix II of the US/Canada bilateral agreement:

1. Stress Adaptations:
    - There were no comments that suggested that any additional specific testing be done to address this issue unless there are unique concerns about the trait/species combination. Case-by-case evaluation within typical variety development programs is sufficient.

2. Plant Hardiness:
    - There is sufficient literature with extensive documentation readily available for this analysis. Most comments suggested that normal variety development testing will address any plant hardiness issues.

3. Effects on symbiont organisms:
    - There were differing opinions regarding the value/need/usefulness of studies on mycorhizae. Current practice in field trials does not look at these interactions. Questions arose about whether the science can effectively address issues given the large number of planting sites, soils, mycorhizal species, etc. that would need to be studied. If the scion is transgenic but not the rootstock, this might not be an issue. In some cases, greenhouse studies are not difficult to do; however, interpreting significant differences and what they mean in an orchard/soil ecosystem may be quite difficult. Management practices alone in an orchard effect mycorhizal populations all the time. Leaf drop from trees expressing anti-fungal or anti-bacterial compounds could affect populations.

4. Life span:
    - Highly unlikely to pose a plant pest risk.

5. Growth habit:

- Current programs are sufficient for evaluation.

Additional comments and questions that came out of the fruit tree group breakout session discussions:

- It is important to separate market risks from safety risks. A risk assessment should address “Is the trait safe?”
- With quality improvement traits where there would be no selective fitness advantage, is there a need for gene flow evaluation?
- A conditional deregulation might be useful in order to limit geographic distribution of transgenic away from wild relatives.
- In determining the minimum number of test sites and years, the genotype by environment variation would likely be so high as to make combined data not very informative.
- Testing in typical production areas may be most appropriate even with environmental effects. This sort of evaluation is useful and important as in a standard breeding/variety development program. Current performance standards, however, restrict wide distribution of transgenic plant materials.
- Although the regulatory relevance of requiring data on fruit quality characteristics was unclear, some of the characteristics might indirectly impact weediness or invasiveness. Fruit quality characteristics are closely evaluated by developers already.
- Rootstock/scion interactions will need some evaluation. Is there translocation of gene products and is this an APHIS regulatory concern?
- Movement of pollen into beehives is likely. Is this (or should this be) a regulatory concern?
- Release of blight resistant American chestnut and plum pox resistant plum may be seen as a significant benefit to address devastating invasive pathogens. A similar example is the release of papaya ringspot virus resistant papayas in Hawaii which has been beneficial for the industry.