Comments on risk assessment of transgenic trees
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Dear Sir/Madam

I wish to offer the following comments and observations as you seek information on risk assessment of transgenic trees. I have studied risk assessment of transgenic trees for approximately 20 years, and two of the background papers for this discussion were in fact produced by my laboratory (DiFazio et al, James et al). You can see my full qualifications here: http://www.cof.orst.edu/coops/tbgrc/Staff/strauss/index.htm

My extensive field research with transgenic trees over the last 15 years, which has included well above 100 USDA APHIS authorized field trials and two permits in the USA, has shown me how costly and onerous the current regulations are for scientific research, even in the USA. Although I support careful, science based risk and benefit assessments of transgenic trees, because I believe that additional stringent requirements for risk assessment are very likely to foreclose most additional field research (and FIELD research is essential for most kinds of reliable risk assessment data), I emphasize the relaxation of regulations and requirements for detailed risk assessment studies in cases where the level of genetic novelty is low.

Because much of the discussion seeks to clarify the specific intents of Annex III, I start by commenting on several of its principles. My comments follow each quoted principle/provision.

1) "General principles /. 5. Risks associated with living modified organisms or products thereof…should be considered in the context of the risks posed by the non-modified recipients or parental organisms in the likely potential receiving environment."

I believe that "context" is the key consideration in risk assessment. Every genotype in a breeding program has some risk of an adverse outcome for biodiversity depending on point of view and to what it is compared. To do a risk assessment without a specific frame for comparison makes no scientific sense. I believe that the CBD should require that all risk assessments be conducted with explicit comparisons to conventional breeding programs and methods, both in general and specific to the tree taxon and environment in question. If a general method is viewed as safe for many crops, such as is chemical mutagenesis, then there is little reason to expect this consequence of the genetic engineering process to be unsafe for transgenic trees. When considering specific novel modifications, it would be appropriate to consider the degree of novelty that is already practiced in breeding programs. For example, a program that makes extensive and legal use of exotic species and inter-species hybrids would have a much higher threshold for

novelty than would a program that only uses local, natural genetic variation. It would not be difficult, for example, to conduct simple chemical or biological assays to determine relative novelty (e.g., by testing a range of genotype from conventional breeding against a panel of herbivores/microbes, and compare it to the effect of a transgenic type).

Where a transgenic modification is clearly less novel than an accepted method of conventional breeding, it would be appropriate to exempt it from regulation entirely. This concept fits with the methodology provision "7. The process of risk assessment may on the one hand give rise to a need for further information about specific subjects, which may be identified and requested during the assessment process, while on the other hand information on other subjects may not be relevant in some instances." Where a modification is modest or similar in comparison to conventional breeding effects, there would be no need to require additional information on its specific effects.

Likewise, for transgenic trees that have modified natural traits based on native or functionally homologous genes (e.g. those for plant metabolic pathways and hormones), a toxicology approach to risk assessment does not make sense, as there is no novel toxin to test. These plants do not have novel toxic properties, just modifications to native plant processes that make them more useful or productive in plantations, similar to the effects of conventional breeding—which have effects on general plant chemistry, but do not introduce major new toxicological novelties.

Under context, it is also important to consider the larger picture, not just specific ecological effects, in risk assessment. For example, if a transgenic tree is more productive and can thus lead to plantings that produce more wood on a smaller area, this can have a large indirect benefit for biodiversity—as has been seen in many countries already. The well known paper by Sedjo and Botkin (1997, "Using forest plantations to spare natural forests," Environ. Health Perspect. 39**:**15-20), made this point powerfully. Clearly, the indirect risks of not using gene technology for its beneficial effects on biodiversity may greatly outweigh most of the direct risks from specific genes and genotypes. I believe that such "opportunity cost" types of risks to biodiversity (e.g., where risk assessment data requirements are so great as to result in loss of technology development) should be explicitly considered in risk assessment protocols adopted by the CBD. This is a serious concern for transgenic trees as many years of field trials, in many places, are often required to generate data, yet field trials are extremely costly and often restricted or impossible due to national regulations. The stringency of regulation for transgenic trees makes this a real programmatic-level environmental risk that should be explicitly considered.

2) "General principles / 6. Risk assessment should be carried out on a case-by-case basis. The required information may vary in nature and level of detail from case to case, depending on the living modified organism concerned, its intended use and the likely potential receiving environment."

The need to tailor data requirements, and regulations for field research, to the specific modifications made is critical. As genomic knowledge of trees progresses, more and more transgenic tree products under development will not contain novel genes or proteins as their "active ingredients," but only native or highly homologous forms whose regulation has been modified. Of course, such genetic variation in regulation of native genes is also extensive in nature. Thus, such modified forms clearly do not require the same scrutiny as, for example, forms with a novel ecotoxic gene from a different kingdom. In fact, many such forms might be exempted from regulation as they are generally BETTER known than the products of many types of conventional breeding, whose gene-level causes of modified properties are unknown or very poorly known. And the variation in the target traits, such as rate of growth or tissue quality, is also extensive in wild samples, which as discussed above should be explicitly considered as context.

This concept fits well with the provision under methodology "8. (a) An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity…" This implies that modifications that are not biologically novel, would require much less scrutiny, or be exempt from regulation. Cis-genic (same species), intra-genic (same genus), and homo-genic (same biological function) types of modifications are examples of classes that might be exempt or in a much reduced scrutiny category.

Note that this suggested fine tuning of risk assessment requirements to the biological risks of different types of cases is a form of case-by-case assessment, however, it also benefits from the recognition of distinct biological categories of novelty and genetic familiarity.

A similar provision would be to exempt, or put in low risk categories, those genes that are foreign to trees but are already in wide use in the environment, and have undergone extensive risk assessment in other plant species. This includes some of the selectable marker and reporter genes, and some promoter and other regulatory elements, already in extensive use in transgenic crops. This point was argued in detail by Bradford et al, (2005, "Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology, and genomics," Nature Biotechnology *23*:439-444). They also discussed how very small releases, and genes with expected domestication effects (neutral or deleterious to fitness in the wild), should be put in low risk or exempt categories, which I also strongly support. Examples are reduced fitness trees as a result of reduced or modified lignin, reduced reproductive fertility, increased reliance on intensive management (fertilization, water) for growth improvement, and reduced height relative to diameter growth.

3) "Methodology 8. (b) An evaluation of the likelihood of these adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism; (c) An evaluation of the consequences should these adverse effects be realized; (d) An estimation of the

overall risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized;"

These provisions emphasize that is the adverse ecological effects, not the simple movement and presence of a transgene, that should be the only focus of risk assessment. Large scale movements of pollen, seed, or vegetative propagules—but that give rise to extremely low frequencies of presence, or have very small ecological impacts compared to conventional breeding and silviculture (with its many genotypes, species, and management/harvest effects)—should explicitly NOT be considered an adverse effect in risk assessments under the CBD, no more than such movement is considered a harm in conventional breeding. Likewise, the possibility of evolutionary increase should not be assumed in risk assessment, given that most genetic manipulations are directed at highly managed environments such as plantations, not at success in the wild, and most genes for biotic stress resistance are unlikely to be sustainable given the rapid relative evolutionary rates of pest populations compared to that of wild or feral trees. In other words, fitness benefits are very likely to be much reduced over a small number of generations for trees, greatly restraining the speed and extent of increase in transgene frequency that might occur in wild or feral trees.

Finally, with respect to small and short term field trials (e.g., below 10 to 100 hectares, below 10 years), the scale of possible release if often so small that the likelihood of adverse impact is extremely low and remote, even if there is a small release of seeds or pollen. However, where provisions for harvest prior to flowering occur, or harvest of trees in the vicinity of the trial occurs (and thus most matings and regeneration), the release is almost nil compared to conventional breeding. Strict regulations of field trials are therefore inappropriate, especially given their critical importance for risk assessment research, as discussed below.

Finally, I wish to stress two points:

Field trials are essential for risk assessment

As alluded to above, Field trials must be enabled, not restricted—because it is only in the field that risks can be evaluated, and the degree of ecological novelty due to a transgene estimated in comparison to conventional causes of ecological variation (selected varieties, silvicultural and harvest practices, etc). The reason field studies are needed is that tree ecophysiology and chemistry is very different in the field compared to the lab or greenhouse. Thus, risk assessments depend on a reasonable, affordable, efficient regulatory framework that permits a great deal of field research. The CBD therefore has a direct interest in seeing that its negotiations do not give rise to regulations that further restrict the ability of participating countries to do large numbers of field trials.

Models are essential for risk assessment.

The combination of field data to calibrate models, and models that take into account realistic details of the environment, genes, traits, fitness, demography, etc can provide very useful estimates of the range of risk possible over decades to centuries. Sensitivity

analysis allows the reliability of the model to be directly assessed. See the work by DiFazio cited below for one example. This allows investigations of risk scenarios that extend far beyond experimental time frames for trees.

OUR PUBLISHED WORK SUPPORTS THESE GENERAL RISK ASSESSMENT PRINCIPLES. ALL PAPERS CAN BE DOWNLOADED HERE: http://www.cof.orst.edu/coops/tbgrc/Staff/strauss/publications.htm

TRANSGENIC TRAITS ARE HIGHLY STABLE IN TREES

Li, J., A.M. Brunner, R. Meilan, and S.H. Strauss. (2008) Matrix attachment region elements have small and variable effects on transgene expression and stability in field-grown *Populus*. *Plant Biotechnology Journal* 6: 887-896

Li, J., A.M. Brunner, O. Shevchenko, R. Meilan, C. Ma, J.S. Skinner, and S.H. Strauss. (2008) Efficient and stable transgene suppression via RNAi in field-grown poplars. *Transgenic Res.*17: 679-694. DOI 10.1007/s11248-007-9148-1

Li, J., R. Meilan, C. Ma, M. Barish, and S.H. Strauss. (2008) Stability of herbicide resistance over 8 years of coppice in field-grown, genetically engineered poplars *West. J. Appl. For. 23*(2): 89-93

Li, Jingyi (2006) Jingyi Li for the degree of Doctor of Philosophy in Forest Science presented on September 20, 2006. Title: Stability of Reporter Gene Expression and RNAi in Transgenic Poplars over Multiple Years in the Field under Vegetative Propagation.

Strauss, S.H., Brunner, A.M., Busov, V.B., Ma, C., and Meilan, R. (2004) Ten lessons from 15 years of transgenic Populus research. Forestry, 77(5) 455-465

Meilan, R., Auerbach, D.J., Ma, C., DiFazio, S.P., and Strauss, S.H. (2002) Stability of herbicide resistance and GUS expression in transgenic hybrid poplars (Populus sp.) during several years of field trials and vegetative propagation. HortScience,37(2) 277-280

MODELS ARE VALUABLE AND USEFUL FOR RISK ASSESSMENT

Brunner, A., J. Li, S. DiFazio, O. Shevchenko, R. Mohamed, B. Montgomery, A. Elias, K. Van Wormer, S.P. DiFazio, & S.H. Strauss. (2007) Genetic containment of forest plantations. *Tree Genetics & Genomes*, 3:75-100 DOI 10.1007/s11295-006-0067-8 (Strauss is co-senior author)

DiFazio, S.P., Slavov, G.T., Burczyk, J., Leonardi, S., and Strauss, S.H. (2004) Gene flow from tree plantations and implications for transgenic risk assessment. In C. Walter and M. Carson (eds.) Plantation Forest Biotechnology for the 21st Century. Research Signpost, Kerala, India. 405-422

Slavov, G.T., DiFazio, S.P. and Strauss, S.H. (2004) Gene flow in forest trees: gene migration patterns and landscape modelling of transgene dispersal in hybrid poplar.. In H.C.M. den Nijs, D. Bartsch, and J. Sweet (Eds.), Introgression from genetically

modified plants into wild relatives, , Pp 89-106. CABI Publishing, Cambridge, MA, USA.

DiFazio, S.P. (2002) Stephen P. DiFazio for the degree of Doctor of Philosophy in Forest Science presented on January 7, 2002. Title: Measuring and modeling gene flow from hybrid poplar plantations: Implications for transgenic risk assessment.

MUTAGENESIS, FAMILIAR TRAITS, DOMESTICATION TRAITS, AND MODIFICATIONS TO NATIVE GENES MERIT EXEMPTION OR MUCH LOWER SCRUTINY COMPARED TO GENES THAT IMPART NOVEL ECOTOXIC PROPERTIES

Bradford, K.J., Van Deynze, A., Gutterson, N., Parrot, W., and Strauss, S.H. (2005) Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology, and genomics. *Nature Biotechnology*, *23 (4)* 439-444

Strauss, S.H. (2003) Genomics, genetic engineering, and domestication of crops. Science, 300 61-62

VERY HIGH LEVELS OF CONTAINMENT ARE FEASIBLE WITH SUFFICIENT FIELD RESEARCH TO DEVELOP AND TEST CONTAINMENT GENES

Brunner, A., J. Li, S. DiFazio, O. Shevchenko, R. Mohamed, B. Montgomery, A. Elias, K. Van Wormer, S.P. DiFazio, & S.H. Strauss. (2007) Genetic containment of forest plantations. *Tree Genetics & Genomes,* 3:75-100 DOI 10.1007/s11295-006-0067-8 (Strauss is co-senior author)

Skinner, J.S., Meilan, R., Ma, C., and Strauss, S.H. (2003) The Populus PTD promoter imparts floral-predominant expression and enables high levels of organ ablation in *Populus, Nicotiana* and *Arabidopsis*. *Mol. Breed.*, 12 119-132

Meilan, R., Brunner, A.M., Skinner, J.S., and Strauss, S.H. (2001) Modification of flowering in transgenic trees. In N. Morohoshi and A. Komamine (Eds.), Molecular Breeding of Woody Plants, , Pp 247-256. Elsevier Science B.V.

EXTENSIVE FIELD TRIALS ARE NEEDED FOR RISK ASSESSMENT STUDIES

Valenzuela, S., and Strauss, S.H. (2005) Lost in the woods. *Nature Biotechnology*, *23* 532-533

Brunner, A.M., Busov, V.B., and Strauss, S.H. (2004) Poplar genome sequence: functional genomics in an ecologically dominant plant species. Trends in Plant Sci, 9:49-56

Strauss, S.H., and Brunner, A.M. (2004) Tree biotechnology in the 21st century: Transforming trees in the light of comparative genomics. In S.H. Strauss and H.D. Bradshaw (Eds.), The BioEngineered Forest: Challenges to Science and Society, , Pp 76-97. Resources for the Future, Washington, D.C., USA.

Campbell, M.M., Brunner, A.M., Jones, H.M., and Strauss, S.H. (2003) Forestry's Fertile Crescent: The application of biotechnology to forest trees. Plant Biotech. J., 1 141-154.

Strauss, S.H., DiFazio, S.P., and Meilan, R. (2001) Genetically modified poplars in context. For. Chron., 77(2) 271-279.