The Regulation of Transgenic Trees in North America

By M. A. McLean[1]) and P. J. Charest[2])

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Abstract

Canada and the United States have both developed strong, science-based systems for the regulation of transgenic plants to ensure environmental protection. In both countries transgenic plants cannot be introduced into commerce unless they have been critically evaluated for environmental safety. The framework for the regulation of transgenic plants in Canada and the U.S. is comparable, however there are significant differences. In Canada, the Canadian Food Inspection Agency is responsible for the regulation of importation and environmental release of plants with novel traits that includes, but is not limited to, transgenic plants. In the U.S., the Animal and Plant Health Inspection Service is responsible for the regulation of importation, interstate movement, and environmental release of transgenic plants. The U.S. Environmental Protection Agency registers certain pesticides produced in transgenic plants prior to their distribution and sale and establishes tolerances for the pesticides in the plants. Details of the Canadian and U.S. regulatory systems are presented, including information on the key criteria utilized in environmental safety assessments, with an emphasis on some unique challenges posed by transgenic trees. To date, the U.S. has authorized the release of one transgenic tree species (papaya) and has allowed approximately 124 confined trials of transgenic trees. Canada has authorized only two transgenic tree trials thus far.

Key words: transgenic plants, transgenic trees, plants with novel traits, regulation, environmental safety, risk assessment.

Introduction

Canada and the United States have each adopted a coordinated approach to the regulation of transgenic plants whereby the regulatory responsibility is shared by several agencies. In Canada, the Canadian Food Inspection Agency (CFIA) has the mandate to regulate field testing and unconfined releases of plants with novel traits, and to assess the livestock feed safety of these plants. Health Canada is responsible for evaluating the food safety of these plants and products, and through the Pest Management Regulatory Agency, contributes to the evaluation of plants with insecticidal properties. In the United States, the United States Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS) regulates the movement, importation, and field testing of transgenic plants. The U.S. Environmental Protection Agency (EPA) regulates transgenic plants with pesticidal properties, and the Food and Drug Administration evaluates food products derived from these plants. Regulatory oversight of transgenic crops began in the late 1980s and since that time both Canada and the U.S. have modified existing legislation to accommodate this new technology.

This paper presents an overview of the regulation of transgenic plants in Canada and the U.S. in terms of their environmental release, and then specifically addresses additional challenges that are unique to the regulation of transgenic trees.

Product vs. Process Based Regulatory Systems

The regulation of genetically engineered[3]) (transgenic) plant species, including tree species, is very similar in Canada and the United States with one significant difference. In Canada, the trigger for assessment is the novelty of the plant rather than the method used to produce it, hence the adoption of the term "plant with novel trait[4]) (PNT)". This means that plants produced using recombinant DNA (rDNA) techniques, chemical mutagenesis, cell fusion and even conventional cross breeding will be subject to regulatory oversight if the introduced trait(s) is considered novel. In contrast, the trigger for assessment in the U.S., and all other countries which have established regulatory systems for transgenic plants, is the method used to introduce the new trait(s). Therefore, in all countries other than Canada, only plants produced using rDNA technologies are subject to this regulation.

The product-based approach to the regulation of novel plants has received widespread support in the scientific community (Tiedje *et al.*, 1989; OSTP, 1986; NAS, 1987) where it is accepted that the environmental impact of a new plant variety is determined by the biology of the plant itself, and not by the technique used to produce it. For example, a herbicide tolerant (HT) plant produced by chemical mutagenesis has the same potential to influence the environment as a transgenic plant expressing the same HT trait.

Interestingly, the regulation of PNTs in Canada was solely precipitated by the process of transgenic plant breeding as the introduction of new plant varieties (with the exception of exotic species) was not reflected as an environmental concern in Canada's regulatory system until 1988 when confined field trials of novel plants were first required.

One of the challenges with using a product-based trigger to initiate regulatory oversight of novel plants is determining when a plant is, in fact, novel. To facilitate this, the CFIA has developed a safety based model for the regulation of PNTs. This model, summarized in *Figure 1*, uses the concepts of *familiarity* and *substantial equivalence* to determine if a plant is a PNT and therefore subject to regulation. In Step 1 of the model, familiarity considers the plant species, the trait(s), the trait introduction method, and whether cultivation practices will be similar to those already in use for that plant species in Canada. Step 2 is designed to evaluate if the plant in question is substantially equivalent to its traditional counterpart(s) and considers five key environmental criteria: altered weediness

[1]) AgBioS Inc., Merrickville, Ontario, Canada K0G 1N0 (mamclean@agbios.com).

[2]) Canadian Forest Service, Natural Resources Canada, Ottawa, Ontario, Canada K1A 0E4 (pcharest@nrcan.gc.ca).

[3]) A technique whereby individual genes can be copied and transferred to another living organism to alter its genetic make up and thus incorporate or delete specific characteristics into or from the organism (Food and Drink Federation http://www.foodfuture.org.uk/index.htm).

[4]) A plant variety/genotype possessing characteristics that demonstrate neither familiarity nor substantial equivalence to those present in a distinct, stable population of a cultivated seed in Canada and that have been intentionally selected, created or introduced into a population of that species through a specific genetic change.

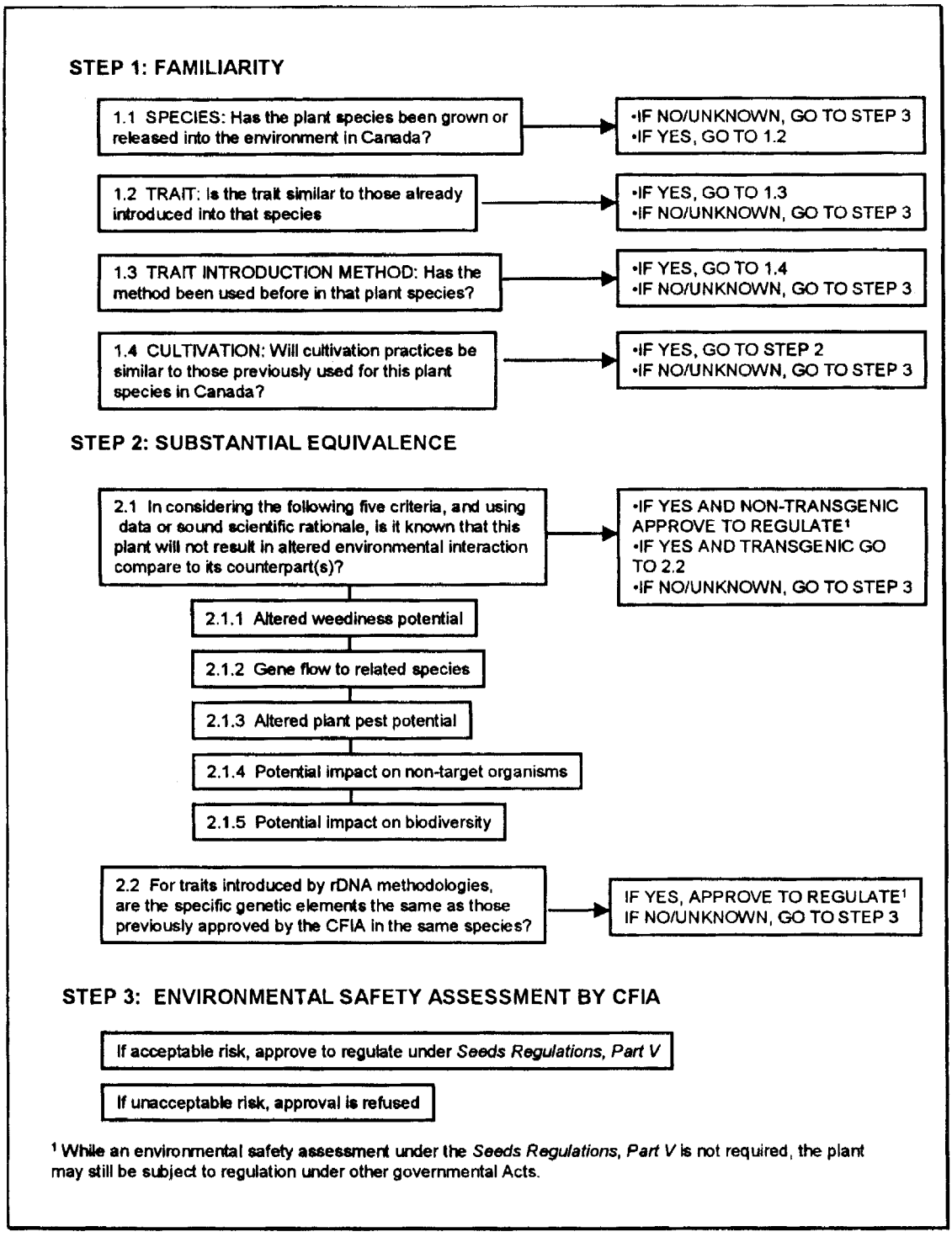

Figure 1. – A schematic representation of the Safety Based Model for the Regulation of Plants (AAFC, 1994).

potential, gene flow to related species, altered plant pest potential, potential impact on non-target organisms, and potential impact on biodiversity.

In contrast, when using a process-based trigger, the method used to produce the plant is the sole determinant for initiating its regulation. Therefore, unlike Canada where the novelty of the trait is driving the requirement for regulatory oversight, all other countries have regulatory systems which presuppose that the methodology used to introduce the trait is the first determinant of any potential environmental risk.

In utilizing a process-based trigger, the U.S. and other countries recognise that there is an established history of environmental safety with plants produced using so-called conventional plant breeding[5]) techniques, but that transgenic plants necessitate an additional level of scrutiny until a similar history is established for this new breeding tool. Although the trigger for assessment in the U.S. (and elsewhere) is rDNA,

[5]) Conventional plant breeding refers to techniques other than rDNA that have been used to produce new plant varieties and that have an established history of safe use. Examples are natural cross-breeding, wide crosses, selection of somaclonal variants, chemical mutagenesis, radiation mutagenesis, embryo rescue, and cell fusion.

APHIS' evaluations are in practice product based as each transgenic plant is assessed on a case-by-case basis.

Movement through the Regulatory Framework

Confined Trials

Transgenic plants are developed and initially assessed in a contained environment such as a laboratory, growth chamber or greenhouse. The next step is to evaluate promising material in experimental field trials that are mandatorily conducted under conditions designed to confine the test plants and restrict any environmental impact. Confined field trials are necessary to evaluate both the agronomic or silvicultural characteristics of the plant, and to provide the developer with the opportunity to evaluate safety by collecting the data required to satisfy the regulatory criteria for an unconfined release (Canada) or deregulation (U.S.). In Canada and the U.S. proponents must apply for permission to conduct field

TERMS AND CONDITIONS FOR CONFINED POPLAR TRIALS

1. The trial term will be limited to eight years from the date of commencement.

2. The transgenic trees will be clearly labelled and will be transported in a safe and secure manner to prevent accidental release. A complete documentation of propagule custody will be kept.

3. In case of accidental release, recoverable plant material will be collected. The site will be marked and monitored (if necessary). The Canadian Food Inspection Agency (CFIA) will be notified.

4. Site maintenance and harvesting machinery will be thoroughly cleaned on-site, prior to being moved to other sites, to prevent dissemination of genetically modified plant material.

5. The boundaries of the trial site will be marked during the trial period and the post-harvest restriction period.

6. Two guard rows will be composed of non-transformed balsam poplar (*Populus balsamifera*) producing no or very few suckers

7. Surplus transgenic trees from the trial will be clearly labelled and kept in a secure greenhouse facility or will be destroyed by mechanical means, heating or burning.

8. The trees will be cut down at the end of the trial period and all precocious inflorescences (if any) will be removed each year before anthesis to prevent pollen and seed dissemination. Records will be kept of the date and number of flowering catkins removed for each genetic line.

9. The trial material (including the guard rows) will be separated by a distance of at least 15 metres from other trees of the same or related species. The trial site and isolation distance will be monitored regularly, at least twice a week during the period of flowering and budburst and every week during the growing season of the trial period to ensure that all suckers, precocious inflorescences and trees of the same or related species that are not part of the trial are destroyed.

10. In case of unexpected spread, volunteer poplar plants and plants arising from vegetative propagation will be mechanically or chemically destroyed.

11. The Plant Biotechnology Office must be notified each year with a written annual report and a final report at the completion of the trial.

12. Plant matter remaining at the end of the trials will be destroyed. Stumps on root systems will either be mechanically destroyed on site or removed and destroyed. The trial site will be tilled and any developing suckers after tillage will be destroyed.

13. The site will not be used to grow poplar trees for five years from the date of termination of the trial. The site will be monitored regularly, at least monthly, during the growing season of the post harvest restriction period to ensure that any volunteer plants and suckers are destroyed.

14. The post harvest restriction period will be extended if suckers are still observed during the fifth year of the post harvest restriction period.

15. Inspectors of the Canadian Food Inspection Agency will have access to data and observation logs. A report indicating whether the trial was run as proposed, including a description of any protocol changes, their justifications and encountered problems will be submitted to the CFIA, Plant Health and Production Division, upon completion of the trial.

Figure 2. – Typical terms and conditions for confined field trials of poplar in Canada (CFIA, personal communication).

trials with transgenic plants, and must abide by the terms and conditions of authorization. Typically field trials must be reproductively isolated, there are restrictions on post harvest use of the land, and current and post-harvest monitoring of the trial site is required. An example of the terms and conditions for confinement of an authorized poplar trial in Canada are presented in full in *Figure 2*. Eligibility criteria and performance standards for confined trials are found in *Regulatory Directive 2000-07: Guidelines for the Environmental Release of Plants with Novel Traits within Confined Field Trials in Canada* (CFIA, 2000) and *7 CFR Part 340* (USDA, 1987).

Environmental Releases

The criteria used to assess the environmental safety of releasing a transgenic plant are very similar in Canada and the U.S. The safety assessment can be divided into two parts:

Table 1. – Transgenic tree species in confined trails in the U.S. (1989-present).

Organism	Trait	Year	Organism	Trait	Year
Apple	Lepidopteran resistant	1991, 1997, 1999, 2000	*Populus deltoides*	Herbicide tolerant	1998, 2000
	Fireblight resistant	1993, 1994, 1998, 1999		Marker gene	1999
	Coleopteran resistant	1995	Hybrid poplar	Marker gene	1989, 1999
	Fireblight resistant, altered fruit ripening	1995		Lepidopteran resistant	1993
	Altered fruit quality	1997		Phosphinothricin tolerant, male sterile	1995
	Altered flowering	1998		Glyphosate tolerant	1996, 1997, 1998, 1999, 2000
	Apple scab resistant	1998, 1999, 2000		Fungal resistant	1997, 1998
	Increased sugar alcohol levels	1999, 2000		Insect resistant: Cottonwood leaf beetle, Phratora leaf beetle	1997
	Oblique banded leafroller resistant	1999		Marker gene, sterile	1997
	Reduced ethylene synthesis	1999		Coleopteran resistant	1998, 1999, 2000
Coffee	Reduced ethylene synthesis	1999		Altered lignin biosynthesis	1998, 2000
	Reduced caffeine levels	1999		Constitutive expression of glutamine synthetase	1998
Grapefruit	Aphid resistant	1999		Cell wall altered	1998
	Marker gene	1999		Crown gall resistant	1998
	Closterovirus resistant	1999		Septoria resistant	1999
Papaya	Virus resistant	1992, 1995		Halogenated hydrocarbons metabolized	1999
	Virus resistant	1997, 1998, 1999	Serviceberry	Lepidopteran resistant	1992
	Reduced ethylene synthesis	1998	Spruce	Lepidopteran resistant	1993
	Virus resistant, reduced ethylene synthesis	1998	Sweetgum	2,4-D tolerant	1995, 1998
Pear	Altered agronomic properties	1999		Glyphosate tolerant	1999, 2000
Persimmon	Drought tolerant	1999		Altered plant development	1999
	Marker gene	1999		Marker gene	2000
	Lepidopteran resistant	1999	Walnut	Lepidopteran resistant	1990, 1991, 1993, 1997
	Fungal resistant	1999		Fungal resistant; virus resistant; Lepidopteran resistant	1995
Pineapple	Virus resistant, altered flower and fruit set, increased fruit sweetness, root-knot nematode resistant	1997		*Pratylenchus vulnus* resistant	1997
Pine	Marker gene	1998, 1999		Altered flowering	1997
Plum	PRV resistant	1992		Bacterial leaf blight resistant	1998
	PPV resistant	1995		Adventitious root formation increased	1998, 2000
	Reduced ethylene synthesis	1999			

1. the molecular characterization of the transgenic plant in comparison with its conventional counterpart; and 2. the environmental impact of the whole plant, again in comparison with its unmodified counterpart.

In 1998 the CFIA, APHIS and Health Canada harmonized their respective regulatory requirements for the molecular characterization of transgenic plants. Details can be found in the *Canada and United States Bilateral on Agricultural Biotechnology Appendix I: Molecular Genetic Characterization Data* (CFIA, 1998).

In Canada, information requirements for the environmental impact analysis of a transgenic plant (or any other PNT) are presented in the *Seeds Regulations, Part V* and *Regulatory Directive Dir94-08: Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits* (AAFC, 1994). *7 CFR Part 340* (USDA, 1987) and the *Guide to Preparing and Submitting a Petition for Genetically Engineered Plants* (USDA, 1996a) describe the information requirements in the U.S. These are summarized below:

Criteria for the Environmental Safety Assessment of Transgenic Plants

Canada

1. Description of the PNT including taxonomy and pedigree of the PNT and details on anticipated use.

2. Description of the modification: method used to introduce the novel trait(s); molecular characterization if transgenic; parental genome if allopolyploid; data demonstrating stability of the novel traits over multiple generations.

3. Description of the novel traits: description and activity of gene products, breakdown products, by-products and their metabolic pathways; tissue and/or temporal specificity; description of inducer (if required); toxicity of gene products, breakdown products, and by-products in the environment, including effects on predators, grazers, parasites, pathogens, competitors and symbionts, and any potential known adverse effects on human health.

4. Biology of the PNT: reproductive and survival biology; adaptation to stress factors; if the gene product is toxic, data is required on the level of exposure and effect on soil micro flora and fauna.

5. Agricultural/silvicultural practices: details on proposed release sites; changes in usual habitat or normal geographic distribution for the plant species; changes in cultivation or management practices; and deployment strategies.

6. Discussion of potential for gene flow from the PNT to related species and details of the consequences of introgression.

U.S.

1. Description of the biology of the nonmodified recipient plant and information necessary to identify the recipient plant in the narrowest taxonomic grouping applicable.

2. Relevant experimental data and publications.

3. A detailed description of the differences in genotype between the regulated article and the nonmodified recipient organism. Include all scientific, common, or trade names, and all designations necessary to identify: the donor organism(s), the nature of the transformation system (vector or vector agent(s)), the inserted genetic material and its product(s), and the regulated article. Include country and locality where the donor, the recipient, and the vector organisms and the regulated articles are collected, developed, and produced.

4. A detailed description of the phenotype of the regulated article. Describe known and potential differences from the unmodified recipient organism that would substantiate that the regulated article does not pose a greater plant pest risk than the unmodified organism from which it was derived, including but not limited to:

a. Plant pest risk characteristics

b. Disease and pest susceptibilities

c. Expression of the gene product, new enzymes, or changes to plant metabolism

d. Weediness of the regulated article and impact on the weediness of any other plant with which it can interbreed

e. Agricultural or cultivation practices

f. Effects on nontarget organisms

g. Indirect plant pest effects on other agricultural products

h. Transfer of genetic information to organisms with which it cannot interbreed

Authorizations

When a proponent has met all of the information requirements, a determination of non-regulated status (U.S.) or an authorization for unconfined release (Canada) may be granted. Canada additionally allows for conditional authorizations. For example, the deployment of crops expressing *Bacillus thuringiensis* (Bt) δ-endotoxins requires the proponent to develop and execute an approved insect resistant management plan designed to mitigate the development of Bt-resistant populations of target pests. If a plan is not implemented then the authorization of the Bt crop may be cancelled. In the U.S. the E.P.A. regulates Bt crops as pesticidal plants and so an authorization additional to APHIS' deregulation is required before these plants can be released. Such authorization may include a mandated management program to mitigate the evolution of Bt-resistant insect populations.

Canada's *Seeds Regulations, Part V* (CFIA, 1997) has a New Information Requirement which states that a person who becomes aware of any new information about a PNT regarding risk to the environment, including risk to human health, must immediately provide that information to the regulatory authorities. A re-evaluation of the PNT will be conducted and its authorization may remain unchanged, may be revoked, or additional or different conditions may be required respecting the release.

The Regulation of Transgenic Trees

As with genetically engineered crop plants, APHIS and the CFIA are responsible for regulating transgenic trees in their respective countries. Both Agencies have the flexibility to use the same regulations and guidelines for trees as they do for crop plants as evaluations for confined trials and unconfined releases are conducted on a case-by-case basis. The CFIA is supported by the Canadian Forest Service, which provides expert advice to the regulatory authorities on issues pertaining to trials and releases of forest trees with novel traits, including transgenic trees.

The U.S. is the global leader in the evaluation of genetically engineered tree species in confined trials *(Table 1)*. To date only two field trials of transgenic trees have been planted in Canada: poplar with a marker gene (planted 1997), and herbicide tolerant poplar (planted 1998).

Trees have many characteristics that make them more challenging to assess than agricultural crops: they are long lived; the production cycle may be 50 to 60 years or longer; pollen movement can occur over enormous distances; intra-specific populations have tremendous genetic and phenotypic variabili-

Table 2. – Conditions for reproductive isolation of five transgenic tree trials permitted by APHIS. From *Environmental Assessment and Finding of No Significant Impact* for Permits 92-191-01 (plum), 93-039-02 (poplar and spruce), 94-039-03 (apple), and 94-306-01 (sweetgum).

Species	Trait(s)	Duration of Trial	Size of Trial	Conditions for Reproductive Isolation
Plum (*Prunus domestica* L.)	Papaya ringspot virus coat protein; α-D-glucuronidase; neomycin phosphotransferase	4 years	36 trees	Plants isolated 660 feet from any plum grown for the production of foundation seed; plants to be planted at least 10 days after commercial plum plantings within a distance of 660 feet from the test plot (temporal isolation); plant tassels bagged prior to pollen shed; one or more rows of nontransgenic borders to provide an additional barrier to pollen dissemination
Poplar (*Populus alba x P. grandidentata*	*Bacillus thuringiensis* Cry1A(a) protein; α-D-glucuronidase ; neomycin phosphotransferase	8 years	120 trees	Plants harvested before sexual maturity; any flowers noted to be removed and bagged and the tree bearing the flowers will be cut down and the cut end of the stump treated with systemic herbicide; all prunings to be air dried 1-2 months, visually inspected before disposal and any sprouts to be treated with herbicide; 10 foot buffer strip to be mowed around planting (including 5 foot wide tilled strip), any shoots observed to be treated with herbicide; isolation distance of 130 m between transgenic poplars and any other nonexperimental plants
White spruce (*Picea glauca*)	*Bacillus thuringiensis* Cry1A(a) protein; α-D-glucuronidase ; neomycin phosphotransferase	15 years	120 trees	Plants harvested before sexual maturity; any flowers noted to be removed or bagged and the tree bearing the flowers will be cut down and the cut end of the stump treated with systemic herbicide
Apple (*Malus domestica* Borkh)	Attacin E or cecropin (giant silk moth) or lysozyme (hen); α-D-glucuronidase ; neomycin phosphotransferase	7 years	<5 acres	Blossom production not required (rootstock) and so will be prevented by pruning practices; rootstocks grafted with scion cultivars will be treated annually for removal of rootstock suckers; non-grafted rootstocks will be trained to a single shoot and cut back every year
American sweetgum (*Liquidamber styraciflua* L.)	2,4-dichlorophenoxyacetic acid monooxygenase; neomycin phosphotransferase	3 years	80 trees	Plants harvested before sexual maturity; any flowers noted to be removed or bagged.

ty; and the inherent ecological complexity that exists in a forest is comparatively absent from, for example, a corn field. Even the concepts of familiarity and substantial equivalence must be re-evaluated, particularly if they are to be applied to forest tree species. What constitutes a distinct, stable population of spruce in Canada or poplar in the U.S.? When comparing a genetically engineered pine to its traditional counterpart, what are the accepted norms for the unmodified tree species? Questions such as these can only be answered through continued research into the biology of key species currently used in transgenic research (eg. poplar and spruce), particularly in managed ecosystems like plantations where deployment of these trees is likely to be limited (STRAUSS *et al.*, 1999). Confined field trials of transgenic and unmodified trees are necessary to achieve this end.

The reproductive isolation of transgenic trees in confined field trials in the U.S. and Canada has been addressed by both APHIS and the CFIA in the same way: all trees under trial (transgenic and controls) must be destroyed before flowering. Specific examples are provided in *Table 2* for five tree species permitted for trial in the U.S. While this approach is effective in preventing pollen flow from test plants, it eliminates the

opportunity for analysing what is arguably one of the most significant and contentious issues around the release of transgenic trees – gene introgression. Large scale field trials of transgenic trees transformed with benign marker genes (eg. green fluorescent protein) could provide a powerful experimental model for studying pollen movement and gene flow but only if the trees are permitted to continue past the point of sexually maturity.

Thus far, the U.S. is the only country to have authorized the environmental release of a transgenic tree. In 1997 APHIS deregulated the transgenic Sunset papaya lines 55-1 and 63-1 which were developed to resist infection by papaya ringspot virus (PRV). The viral coat protein gene from a mild strain of PRV was introduced into the genome of Sunset papaya via *Agrobacterium*-mediated transformation as part of a genetic construct that also included the *nptII* and *uidA (gus)* marker genes. More information about this release, which is limited to a very small geographic area, is available in APHIS' published decision document (USDA, 1996b).

At present, there are no other petitions for the deregulation of transgenic trees pending. *Tables 1* and *2* show that, unlike traditional agricultural crops, the time line in moving from development through evaluation, and perhaps approval is significantly expanded for tree species. Just how long is long enough to accrue sufficient data for an environmental safety assessment remains controversial.

Conclusion

Canada and the United States have robust, science-based regulatory systems that have proven effective in providing for the environmental safety of field trials and unconfined releases of transgenic plants. The case-by-case approach to product evaluation is well suited to accommodate the unique challenges posed by the assessment of transgenic tree species. Both regulatory systems have the flexibility to effectively evaluate the environmental safety of transgenic trees and remain responsive to scientific development and innovation.

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Random Amplified Polymorphic DNA (RAPD) Analysis of Genotypic Identities in *Eucalyptus* Clones

By M. L. DE LAIA, E. A. GOMES, E. J. ESBRISSE and E. F. DE ARAÚJO[1])

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Summary

Vegetative micropropagation is usually applied in *Eucalyptus* in order to obtain clones for improvement on plant propagation for commercial purposes. One problem of this technique is somaclonal variation, which serves as a source of undesirable genetic variation, in a propagation of previously selected clones. To analyze the genotypes, *Eucalyptus* clones hybrids obtained by vegetative micropropagation were evaluated by RAPD markers. Fifteen arbitrary 10-mer primers were successfully used to amplify DNA of four clones obtained in different subcultures from callus to adult plants. During the analysis of clone "A" polymorphism was observed in the pattern of fragments of amplified DNA among subcultures, producing 39 polymorphic and 23 monomorphic bands. The genetic distance varied from 0 to 37% within this clone. For clones "B", "C" and "D" no polymorphism was observed in all plants in different ages. These results suggest the existence of sample exchange or somaclonal variation in clone "A" and showed that RAPD markers are an efficient tool for the early analysis of genotypes in *Eucalyptus* clones.

Key words: Random amplified polymorphic DNA, RAPD, genotypic identity, *Eucalyptus* clones, somaclonal variation.

[1]) MARCELO LUIZ DE LAIA, ELIANE APARECIDA GOMES, ÉDER JAIME ESBRISSE and ELZA FERNANDES DE ARAÚJO, Universidade Federal de Viçosa, Departamento de Microbiologia / BIOAGRO, Viçosa - MG - Brazil - 36571-000
Fax: +55-31-899-2573
Corresponding author: ELZA FERNANDES DE ARAÚJO
e-mail: ezfa@mail.ufv.br