# RISK-MANAGEMENT ISSUES FOR GENETICALLY ENGINEERED FOREST TREES

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## ABSTRACT

Use of genetic transformation, as the most widely pursued form of "genetic engineering", is often seen as entailing various biological risks. Prominent among the perceived risks for forest trees is genetic contamination of wild populations. However, this cannot happen with exotic species with no local relatives; moreover, suppression of reproduction is desired on independent grounds. Potentially the most serious, yet largely unrecognised, risks for plantation forest species arise from side-effects of genetic transformation on fitness in the field. While these risks may involve low-probability events ("disasters"), such disasters could be catastrophic, except under extremely short rotations, calling for risk management. The risks that are associated just with gene insertion are in principle readily controlled. More problematic are the risks associated with side effects of alien gene (transgene) action interfering with crucial functions such as disease resistance.

Quantitative analysis of such risks was made assuming a range of arbitrary probabilities associated with individual transgenes and interactions among different transgenes within the same recipient genotype, showing how such risks might increase markedly as more transgenes are inserted. More crucial, though, may be the need to avoid over-dependence on insertion of any particular gene, despite the associated risk being of low probability, because of the worst-case potential for dire economic consequences. This approach, however, may be difficult to reconcile with the desideratum of suppressing all sexual reproductive activity. Field testing of transformants will be crucial, as it will eliminate many transformation-related risks. The elimination, however, will be slow and never quite complete, which argues for long periods of applying risk spread. In New Zealand a gap exists in the regulatory provisions for risk management with transformants.

**Keywords**: genetic engineering; transgenics; genetic transformation; risk management; *Pinus radiata*.

## INTRODUCTION

Genetic engineering is now a topic of intense interest for genetic improvement of domesticated crops. It typically entails genetic transformation, the insertion of specific genes or sets of genes. This is pursued in order to create gene combinations that cannot readily arise from natural modes of reproduction, or to selectively overexpress some genes, or inactivate (in whole or in part) genes of unwanted function. However, even when this is achieved, various risks will accompany its implementation. The spectrum of such risks will depend on

the nature of the organism concerned, the nature of the genetic transformation (in terms of the identity of the inserted gene(s), the individual insertional event, and possibly the insertion site and the method of insertion), and the management system under which the organism is grown.

The interest in genetic engineering extends to forest trees, in which a number of experimental genetic transformations have already been achieved (e.g., Walter *et al.* 1998). The long rotations and long generation times may pose problems for this approach, but create for it some special attractions. The issues relating to benefits and the risks of using genetically engineered ("transgenic") forest trees have been reviewed by Mullin & Bertrand (1998). In the course of that review, they covered the customary perceptions of the risks involved, and discussed what might constitute an appropriate regulatory framework for addressing the risks.

With forest trees, genetic contamination of wild populations is widely perceived to be one of the main risks (Carson *et al.* 1997; Mullin & Bertrand 1998), particularly as many trees are wind-pollinated, which greatly increases the problems of containment. By contrast, potential food contamination, which is a politically very sensitive issue with agricultural and horticultural crops and also with edible animal products, is largely a non-issue with forest trees. Other categories of risk that have been cited for genetically engineered plants in general (Carson *et al.* 1997; Reynolds 1997; Mullin & Bertrand 1998; Royal Society 1998) include: spread of herbicide resistance into weed populations, transformant cultivars becoming weeds in their own right, non-durability of genetically engineered resistance to pests or diseases, and even allergenicity or toxicity in transformants.

However, a risk category that has received much less attention comprises **adverse side effects of transformation on crop fitness**, quite apart from possible impermanence of benefits. Aspects of crop fitness that might be affected include resistance to diseases or pests, tolerance of environmental stresses, and, in forest trees, the mechanical stability of standing trees. Since crop fitness involves the security of cultivar crops it could be much more important with forest trees than with most other plants, because of the time for which crops must be grown. Indeed, it could be by far the most important associated risk category for forest trees. While I will review some other categories of associated risks I will focus mainly on this one, with special reference to the circumstances surrounding the cultivation of species such as *Pinus radiata* D. Don.

There could, in addition, be more indirect risks, relating to opportunity costs and management challenges, but these are given only brief mention.

Risks of adverse side effects on fitness are the subject of preliminary quantitative modelling in this paper, assuming a range of low probabilities for the risks associated with both individual transformations and interactions between different introduced genes ("transgenes") that may be introduced together. However, the implications of very serious if low-probability outcomes are considered as a separate issue. Potential avenues for managing the risks associated with genetic transformation of forest trees are considered. I will also review briefly how these avenues fit with the institutions of the forestry sector and the regulatory mechanisms in New Zealand.

In connection with potential adverse effects of genetic transformation on crop fitness, risk management cannot usually be about addressing known probabilities, but rather about major

uncertainties and thus about forearming against the unknown. To a large extent the choice of a quantitative model has to be arbitrary, in respect of both the choice of parameters and the input values of the parameters. However, the parameter values need to be varied widely enough to be likely to bracket true values. Beyond any quantitative assumptions that must be made, considerable surmise has to be accepted. It is necessary to make educated guesses as to where appreciable risks may arise and how they might be mitigated, but surmise should always be explicit so that management approaches can be refined in the light of better knowledge.

Risks associated with genetic engineering must be considered in relation to the prospective genetic gains from transformation, given that the pursuit of high gains tends to incur increased risks. The acceptable level of risk (risk being a function of both the probability and the seriousness of an eventuality) will depend on the level of gain associated with the procedure. The risks concerned must also be considered in a broader context of risk management, which entails actively countering known and present risks, adopting risk spread, and preparing responses against hazards that might eventuate in the future. It must also be realised that measures designed to counter certain risks, which will include genetic engineering, can incur risks of their own, and some such examples will be mentioned in connection with genetic transformation.

## REVIEW OF RISK CATEGORIES

### Genetic Contamination

Risks of genetic contamination are significant not just for their possible impact on wild ecosystems but also for how they could potentiate, or at least accentuate, other categories of risk. Genetic contamination can most readily arise with the spread of pollen from transformant cultivars, particularly with wind-pollinated species, but seed dispersal could also be significant. Widespread concerns have been expressed over possible contamination of wild populations, which could either compromise natural fitness, or introduce some components of fitness (e.g., herbicide resistance) that would increase a species' weed potential (cf. Royal Society 1998). With species that are grown strictly as cultivated exotics, with no close relatives around, genetic contamination is hardly an issue. Producing stocks that can escape as invaders, with the fitness advantage of resistance to some herbicide and/or disease or pest would, however, be very unwelcome.

However important or unimportant the contamination problems may be, suppression of pollen and seed production may, at least for some wind-pollinated species, be set as a regulatory prerequisite for the release of genetically transformed material. As a major bonus, such suppression is also potentially a powerful means of improving effective productivity by stopping the usual diversion of a significant proportion of primary production into unharvestable biomass (Burdon 1995, 1997; Walden *et al.* 1997). As a further bonus, the elimination of reproductive activity (or at least pollen production) could vastly improve the prospects of maintaining open-pollinated *ex-situ* genetic resources of forest trees in an uncontaminated form. This is very hard to assure for *P. radiata* in New Zealand, given the massive use of wind-pollinated material that has been improved by conventional breeding and the consequent contamination pressure that it imposes on gene-resource plantings (Burdon 1995, 1997). By preventing such pollen contamination, the use of genetic transformation can have a very positive impact on the maintenance of natural biodiversity.

## Herbicide Resistance

It is widely believed that transformation may allow the use of more environmentally benign herbicides (Walter *et al.* 1998), although any such use may eventually be negated by mutation and natural selection in weeds (Ellstrand & Hoffman 1990). At the same time, the potential transfer of resistance genes to weeds is likely to depend on intercrossability between the crop and weed species. That is very unlikely with an exotic such as *P. radiata* in New Zealand, and in any case should be precluded if there were a regulatory requirement for suppressing reproduction.

## Pest and Disease Resistance

Both these attributes are seen as potential goals of genetic transformation (Mullin & Bertrand 1998; Walter *et al.* 1998). However, unless the deployment of transgenic material for these attributes is managed well, forest growers may incur relatively high risks of the resistance being overcome by adaptive genetic shifts on the part of the pests or pathogens. The introduction of single genes of large effect, while technically the most straightforward approach, would incur the greatest such risks, because resistance conferred by such genes can be especially vulnerable to single pathogen mutations (Pimentel *et al.* 1989). These risks may often be exacerbated by the length of normal crop life (i.e., rotation length) in many forest trees, although they may be mitigated by such factors as susceptibility being confined to the juvenile stages of tree growth. Arguably, the most insidious risks would attach to the "transgenic" resistance encouraging planting on sites where success is dependent on the material having resistance in a durable form. The introduction of multiple transgenic resistance factors (i.e., "pyramiding" the resistance factors), while promising more durable resistance, would require much greater effort, and may introduce some additional risks of its own, which will be addressed later. A prudent compromise would seem to be to use genetically engineered resistances primarily as a supplement to the resistances that are readily available through conventional breeding. Even better, if some natural resistance exists, would be to further strengthen the breeding work by detection of Quantitative Trait Loci (QTL) using DNA markers which, by helping to ensure the presence of multiple, naturally occurring, resistance factors, promises to give still greater and more durable resistance (cf. Burdon 1995; Gardiner *et al.* 1997).

## Toxicity and Allergenic Effects

Toxicity or allergenic effects could arise through using genetic transformation to confer heartwood durability, to simulate natural durability rather than relying on artificial preservatives. While the natural extractives in wood that confer natural durability may have a "clean green" image, they are often quite hazardous substances, such that occupational health problems arise in the processing of a number of woods. In this light, the judicious use of artificial preservatives may still be a much better option than is often perceived, and *P. radiata* is very easily treated with preservatives. Wind-borne pollen may have some allergenic significance (Fountain & Cornford 1991), but that is most unlikely to be materially accentuated by genetic transformation, while any such problem would be automatically averted by suppressing reproduction.

## Side Effects on Cultivar Fitness

Adverse side-effects could arise from various aspects of the total process of transformation, and may affect climatic tolerances, edaphic tolerances, or disease or pest resistance. For *P. radiata* in New Zealand I would see quirkish effects on disease resistance as potentially the most significant problem, largely because the relatively high, year-round rainfall prevailing in large areas of the plantations is conducive to fungal diseases (Burdon in press). Whatever aspect of crop fitness is affected, the possible adverse side-effects could take several forms (cf. Pimentel *et al.* 1989), notably:

(1) Various classes of adverse somaclonal variation, or cultivar decline, associated with the *in vitro* propagation that is a prerequisite for achieving the genetic transformation;

(2) Adverse genetic changes arising at insertion sites for the transgenes, through disruption of the function of existing genes;

(3) Adverse effects of the introduced genes, through their protein products disrupting some important metabolic function;

(4) Adverse side-effects of eliminating unwanted gene functions (e.g., reproductive activity).

Empirical field testing of transformants prior to operational deployment will remain a basic precaution but, for reasons that are discussed later, may not guarantee absolute protection.

### *Adverse somaclonal variation arising from* in-vitro *culture?*

Somaclonal variation represents persistent variation that can arise among different propagation lines, or subcultures, of a clone. Such effects could be truly genetic, in the sense of involving changes in amino acid codings for functional genes (i.e., classical mutations), or they could be merely epigenetic (e.g., in the nature of persistent maturation or "physiological aging" effects). While somaclonal variation is well known in some plants that are subject to traditional modes of vegetative propagation (e.g., grapes), it appears to be much more prevalent under the *in-vitro* propagation that genetic transformation would entail (e.g., Chen & Ahuja 1993; Fourré *et al.* 1997). Yet, although it can readily arise in *in-vitro* propagation of plants, somaclonal variation does not seem insuperable. Optimising *in-vitro* culture conditions is clearly important. Reducing the times that material is maintained *in-vitro* would help greatly. Maintaining subcultures in parallel, and monitoring and culling for any sign of cultivar decline, which are basic components of good management of vegetative material, should also be valuable precautions. Moreover, "epigenetic" effects of *in-vitro* culture may not be universally adverse; a degree of accelerated maturation (or "physiological aging"), which can occur with *in-vitro* culture in pines (Smith 1986; Frampton & Isik 1987), can be expected to give a much more desirable growth habit in *P. radiata* (cf. Spencer 1987; Menzies *et al.* 1988; Burdon & Miller 1992).

Whether genetic transformation promotes somaclonal variability, beyond what results from *in-vitro* culture associated with transformation, is conjectural.

### *Disruptive effects of gene insertion?*

Genetic transformation usually takes the form of insertion of genes at seemingly random sites in the genome. Such a process is likely to disrupt the function of genes at such sites, if the insertions are within coding regions. While over 95% of the genome of *Pinus* is non-

coding, suggesting that a high proportion of insertions may occur in such regions, it is not certain that genes inserted there will be properly functional. Disruptive gene insertions, however, will almost always affect only one of a pair of alleles in a diploid plant, and actual losses of gene function are usually recessive, or at least largely so. Nevertheless, with forest trees (which are usually diploid) this sort of buffering would typically be less than with those agricultural or horticultural crop plants that are polyploid.

Despite the hope that the disruptions at insertion sites will be recessive and infrequent, and the likelihood that many of the deleterious effects will show up relatively early in the screening process, it seems prudent to spread the risks. That appears readily possible by using a number of independent insertion events in a transformed clone. While the multiple gene insertions may entail much additional work, that should not be prohibitive if the techniques of genetic transformation are refined to the point of becoming routine.

Another obvious precaution is to carry out a transformation on a number of recipient cultivars which will continue to be used in parallel. Maintaining such genetic diversity is typically one of the most basic defences against crop vulnerability, upon which other requisite defensive measures need to be superimposed.

*Disruptive effects of the action of introduced DNA?*

New genes, producing novel metabolites (*see* Category 3 above), or else genes that block unwanted functions such as reproductive activity (*see* Category 4), could conceivably act to disrupt the metabolism of recipient organisms independently of any disruption of gene action at insertion sites. Metabolic disruption, if it occurs, will often produce transformant lines that will rapidly be culled. However, the experience with southern corn leaf blight in the United States in 1970 raises the possibility of a much more dangerous type of outcome. Having flared up on a very limited scale in 1969, the blight emerged as a serious disease in 1970 almost throughout the significant maize-growing areas of the country (Ullstrup 1972). As it emerged later (Levings 1990), the use of the Texas cytoplasmic male-sterility factor for the production of hybrid corn had led to a dramatic breakdown of the resistance to a particular strain of the rust fungus (*Bipolaris* [syn. *Helminthosporium*] *maydis*). Ironically, the long time it took (nearly 20 years) for the particular rust strain to appear spontaneously in United States made the problem far more acute, because of the build-up over time in dependence on the particular factor. This sort of time lag could make such a problem quite catastrophic if it similarly affected pine plantations, because it could affect a very broad age-class, not just the sowings of a single year. Admittedly, the genetic technology involved is now many years old, involving an organelle genome rather than insertion of a specific gene into the nuclear genome, and as such was not the same as genetic transformation as defined here. Nevertheless, what it initially achieved was close enough to what is often being pursued with the new technology for this experience to seem a highly relevant illustration of potential risks.

Selective silencing of introduced genes, confining their activity to certain tissues or certain stages of the tree's life cycle, will demand more sophisticated technology, but should reduce risks of highly adverse side-effects. It may also be important for minimising relatively subtle energetic costs incurred through action of introduced genes.

## Additional Risks

These are some risks that are much less direct (Burdon 1992). For instance, new gene technology potentially carries major opportunity costs if its pursuit entails shifting resources

away from classical breeding activities. Also, the use of new technology is vulnerable to technical obsolescence, and to what one might call "management risks" which relate to possible mismanagement of the development and application of the technology. On the other hand, the opportunity costs may be spurious if the gene technology attracts investment that would, for whatever reasons, not be forthcoming for conventional breeding. The technology is attracting a lot of talent, and there is scope for excellent synergism between genetic technologists and traditional breeders, and among gene technologists working with a wide range of different organisms.

While biological risks may be perceived as the prime concern, they must be seen in a broader context. For instance, it makes little sense trying to reduce them to zero if there are other irreducible risks (e.g., geophysical ones) that are appreciable.

## QUANTITATIVE CONSIDERATIONS

I will address the probability of a disaster resulting from some side-effect(s) of a particular genetic transformation (i.e., the insertion of a specific gene). A disaster is defined as an outcome exceeding a certain threshold of undesirability, although the severity beyond the threshold can vary.

In forest trees the probability of a disaster resulting from adding any transgene is undoubtedly low, but the potential significance is far too great to ignore in a risk management strategy. I see two main aspects as needing to be considered:

- The increasing probability of a disaster according to the number of transgenes incorporated jointly into the same recipient genotype;
- How serious the disaster could be, as a matter to be considered in shaping, and even dominating, a risk management strategy.

The probability of such a disaster is the aspect considered in the following section.

### The Scenario

Consider transformation of n different transgenes, made concurrently on a genotype, designating the individual transgenes 1,..i,..j,..k,..n,

$p_i$ = probability of a disaster occurring just as a result of transgene i*,

$p_{ij}$ = probability of a disaster occurring through a first-order interaction resulting specifically from the combination of transgenes i and j ($i \neq j$),

$p_{ijk}$ = probability of occurrence through a second-order interaction resulting specifically from the combination of i, j, and k ($i \neq j \neq k$)

and so on, until we have

$p_{1...ijk....n}$ = the probability associated with the $(n - 1)^{th}$-order interaction involving all n transgenes.

A general quantitative formulation for the probability (P), for such an disaster occurring with n transgenes is as follows:

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* Note that while this is formally addressing a "main effect" of transgene i, it could effectively represent some interaction(s) between transgene i and one or more genes in the recipient genome.

$$P = 1 - \prod(1 - p_I) \quad (1)$$

where $\prod$ denotes the product of the various $1 - p_I$ for all the cases of $p_I$ concerned,

$p_I$ denotes the probability of a disaster, with potential disasters relating to all the n transgenes in themselves, and to all the interactions, of orders 1 to n –1, between different transgenes.

With almost no hard information on the level of any type of risk, and with the interest being in the general behaviour of likely risk levels, some simplifying assumptions are deemed appropriate:

- all $p_i$ identical, i.e., $p_i = p_j = p_0$
- likewise, all $p_{ij}$ identical, i.e., $p_{ij} = p_{ik} = p_1 \quad (j \neq i)$
  and so on for the increasing orders of interaction
- p decreasing according to order of interaction, such that $p_i > p_{ij} > p_{ijk}$, etc., arbitrarily according to a constant ratio, s (<1), thus representing a pattern of exponential decrease according to the level of interaction, s being the "decay" parameter.

Thus p for the increasing orders of interaction (q) is denoted by

$$p_q = p_0 s^q \quad (0 \leq q \leq n - 1) \quad (2)$$

On this basis, Equation 1 can be rewritten as

$$P = 1 - \prod_{q=0}^{q=n-1}(1 - p_0 s^q)^{**} ({}^nC_{q+1}) \quad (3)$$

where $q = 0$ denotes the simple case of the probability of a disaster arising purely from a single transgene,

$q = 1$ denotes the corresponding probability arising from a first-order interaction,

$q = 2$ denotes that arising from a second-order interaction,

through to the single interaction of order $n - 1$, and

${}^nC_{q+1}$ denotes the number of combinations involved in the various orders of potential interaction, which is given by $n!/[(q + 1)!(n - (q + 1))!]$.

Values of P were calculated according to Equation 2, with the input parameters n, $p_i$, and s varied factorially, with n ranging from 1 to 30, $p_i$ having arbitrary values of 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005, and 0.0001, and s having arbitrary values of 0.5, 0.25, 0.1, 0.01, 0.005, and 0.001.

## Results

Results are summarised in Fig. 1. With the higher values of p (0.1 and 0.05) the value of P (the probability of a disaster) rose rapidly with increasing n. The role of interactions, governed by the parameter s, was such that at those values of p, P continued a rapid almost linear approach towards unity with the higher values of s, such that $P \rightarrow 1$ with $n \approx 10$. At the lower values of p ($\leq 0.01$) the potential role of interactions became much more important, reflecting the sheer numbers of potential higher-order interactions rather than high probabilities associated with any one interaction. The importance of numbers of potential interactions is most clearly evidenced by the way in which the rise in P with n was initially very slow, only to climb rapidly thereafter for the higher s values at these levels of p. The significance of the value of s was particularly evident at low p. Decreasing s below 0.01 had very little impact on P, which means that interactions have almost no expected importance below this value of s.

At all levels of p, there were values of s that gave $P \rightarrow 1$ well within $n = 30$. There is thus a considerable range of conditions under which P can be well above zero, if not approaching unity.

## DISCUSSION

### The Quantitative Model and its Assumptions

The quantitative model addressed, while a classical risk-assessment model, was inevitably simplistic, partly in the interests of tractability and partly in recognition of the present lack of specific knowledge. It was devised to illustrate the potential behaviour of the system under a wide range of scenarios, rather than to serve as a ready-to-use decision aid. With very imperfect information, however, risks will tend to be given greater weight.

An important simplification was the assumption of basic independence of probabilities associated with different effects, except for arbitrary low probabilities of disaster associated with the various possible interactions between the effects of different transgenes. Interdependences can often be quantified and modelled where the causal relationships are known. In certain situations, e.g., engineering or aviation, it is well appreciated that whereas various events (mishaps) may not by themselves cause disasters certain combinations thereof will guarantee disaster. In the present context, however, we have essentially no prior knowledge of what combinations of transgenes will interact in such a way. It was therefore deemed appropriate to assume that all interactions of a given order incurred a single average probability of disaster. While those assumed probabilities might be low, this may be effectively compensated for by the potential higher-order interactions being so numerous. Conjecturally, any higher-order interaction, if manifested, might tend to be more serious. However, as noted earlier, even the side-effects of a single transgene might represent a complex interaction involving a number of existing genes within the genome. The final ingredient of a disaster could well be an external event, such as a pathogen mutation. As mentioned earlier, a long delay before such an external event occurred could actually make the disaster much worse in a forest tree crop. In any case, an adverse interaction among transgenes need not necessarily conform to a model of progressive accumulation of identifiable mishaps.

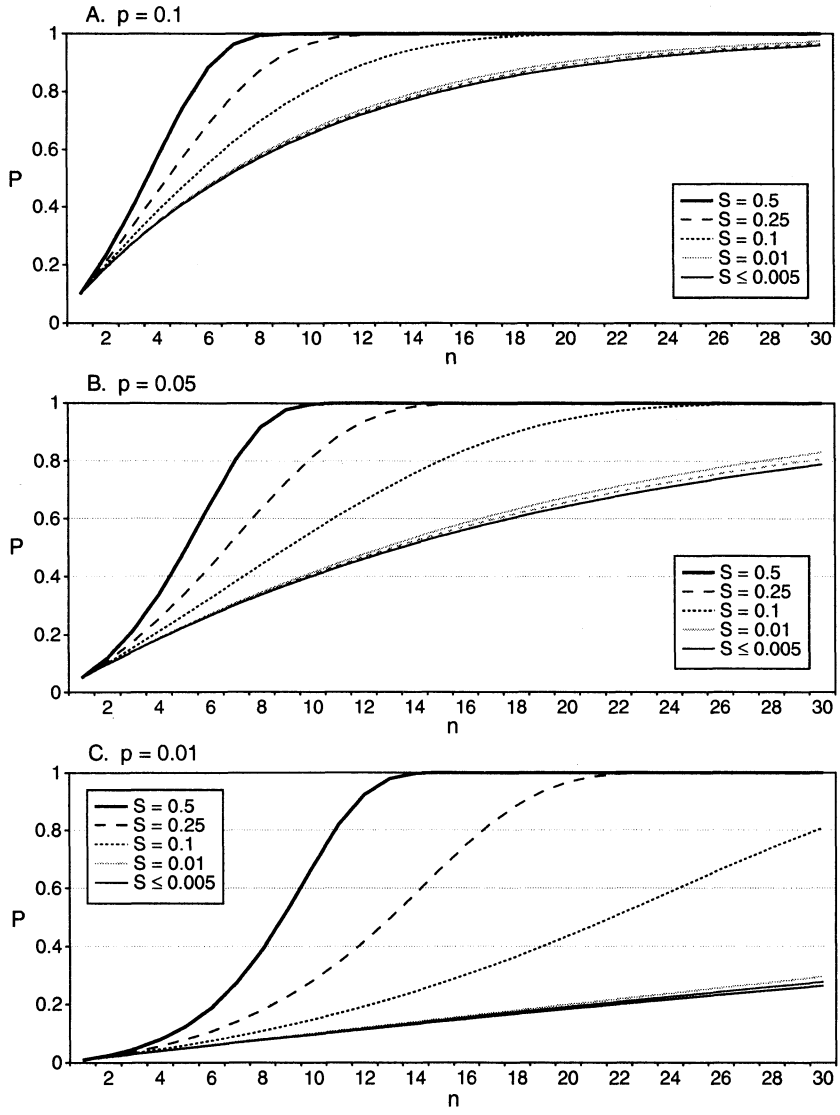
With better knowledge of the biological processes such risk analysis could, in principle, be greatly refined by explicit application of set theory, but this may require great advancement of knowledge.

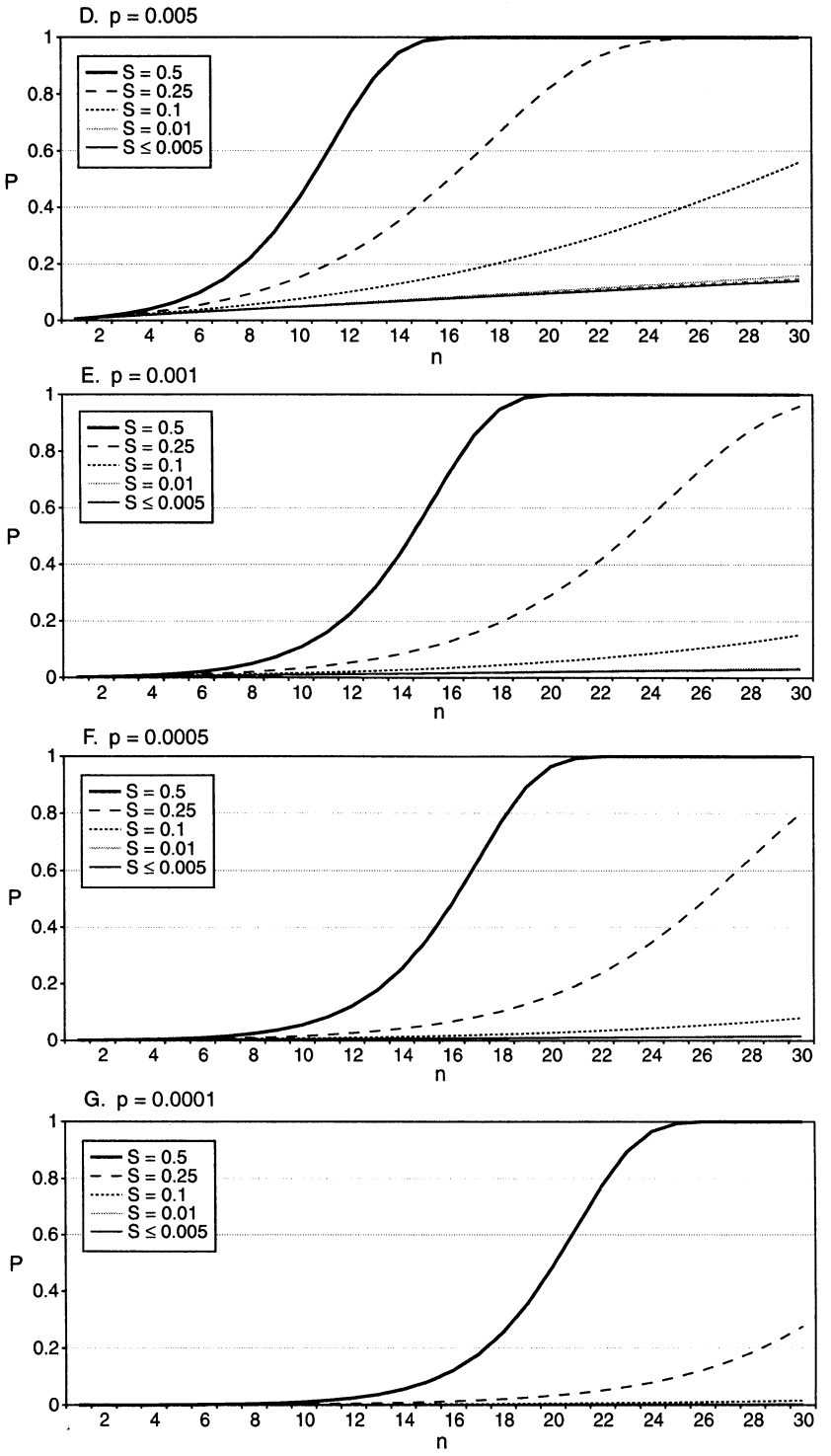
It has also been assumed, for purposes of calculation, that the risks are the same for all introduced genes, and for every combination of a specified number of such genes. In practice, the risks will surely vary according to the type of gene insertion, e.g., between new structural genes and genes that regulate existing genes. And genes introduced from closely related species, for instance, seem likely to entail lower risks than genes that are more fully alien.

Whether transformation introduces a new function or, at the other extreme, blocks an existing unwanted function, may be relevant. In fact, the corn blight crisis involved the elimination of a function, albeit through a spontaneous organelle mutation. To eliminate a function, say, by use of anti-sense DNA, one might target transcription factors like homeotic genes to achieve the desired effect; against that, however, the roles of such genes may be so fundamental that unwanted side-effects of using this approach could be a very significant

risk. In this connection, the elimination of flowering may pose a special problem. As both a possible regulatory requirement and a desideratum for increasing commercial yield, it is a key application of genetic transformation. Unless the associated risks can be minimised, either by using transformations that are known to be "safe" or by using a number of non-flowering transformations that involve quite independent developmental pathways, it will be very hard to assure an appropriate risk spread.

FIG. 1 (*below and facing page*)–Probability (P) of a "disaster" *versus* number of transformations (n), for various levels of p (probability relating to a single transformation) (Parts A to G), and S (parameter linking p to probability of a disaster resulting from interaction between different transgenes).




D. p = 0.005
E. p = 0.001
F. p = 0.0005
G. p = 0.0001
S = 0.5
S = 0.25
S = 0.1
S = 0.01
S ≤ 0.005
P
n
0
0.2
0.4
0.6
0.8
1
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30

Insertion of multiple transgenes involves some additional technical issues that have not been addressed in the model. With present technology such transformations would need to be done by inserting the various genes together in a construct. That would also facilitate spread of risks among different insertional events but, by itself, it could leave exposure to the phenomenon of position effects which could result from particular juxtapositions of genes within the multi-gene construct. Spreading the risk among multiple insertional events would, incidentally, reduce exposure to possible position effects between inserted genes and existing ones. If genes are inserted independently of each other, then either the total number of insertions for all the genes concerned will be so high as to introduce a further risk factor, or it may not be possible to achieve additional risk spread through replicating insertions of particular genes.

The quantitative analysis has addressed only the probability of a disaster, as distinct from the seriousness, which will be discussed below. It is clear that the probability can climb rapidly with inserting a multiplicity of genes into the same individual, and that the rate of climb will also depend strongly on the values of both p and the ratio s. This pattern is significant, because relying on multiple transgenes may be difficult to avoid, although n = 30 is beyond any number that is likely for the time being. There can be several reasons for wanting to insert multiple transgenes into the same genotype, despite the potential increases in risks. These may include: multiple desiderata, involving quality traits to be improved or known risks to be countered; a need for multiple resistance factors to assure durable resistance to a disease or pest; and possible regulatory requirements for reproductive sterility in any transformant material that might be released for operational deployment.

## Seriousness of Unwanted Side Effects

The seriousness of any disaster is governed by

- the severity of the effect,
- the economic importance of the crop (i.e., the particular transformant cultivar), and
- the speed and ease with which an affected cultivar can be replaced either with another cultivar of the same species or with a substitute crop.

The problems of replacement will be acute with a relatively long-rotation plantation crop, dedicated primarily to producing solid-wood products, on land where intensive cultural intervention is often difficult. This is so with *P. radiata*, despite its rapid growth. Crop replacement, however, may be much less of an issue with very short-rotation pulpwood crops, e.g., of eucalypts; against that, capital-intensive processing plant may depend on such crops.

Despite the rise in risks that may be associated with multiple insertions, the worst-case hazard associated with a single, very dangerous effect may need to dominate the risk management. With a long-lived forest plantation crop the key defensive measure may be never to commit too large a proportion of the total crop to insertion of any single gene, unless the biological safety of that insertion is well confirmed. That will, incidentally, reduce the "exposure" to a large array of potential bad interactions among various inserted genes. How large a proportion of the crop can be prudently committed may depend on a number of things, including the length of the rotation, the feasibility of salvage harvesting, and a judgement as to the relative likelihood of trouble.

Where prospective disasters are less serious (e.g., through rotations being very short) the way in which the probability can rise with multiple transformations becomes much more relevant.

## Testing and Deployment Considerations

Field testing of transformants will progressively reduce the risks associated with their use. In practice, it may be a slow process that will never be quite complete. While a significant onus of proof rests upon ensuring biological safety this has, in effect, the problems of trying to prove a null hypotheseis. The corn blight example illustrates how a biological hazard could take many years to manifest itself. Specifically in forest trees, in which transformations conferring substantially different wood properties may be pursued, one should demonstrate the lack of adverse side-effects on the mechanical stability of the trees, which will take a number of years. Although empirical field testing will indicate a progressive downgrading of the risks, some ongoing need for risk spread is seen, and a large element of subjective judgement will persist. Short-cut screening of transformants is attractive in principle, but initial validation will be slow, and it can hardly be targeted against unknown hazards. While transformation may confer certain attributes far sooner than classical breeding, the safe capture of genetic gain will be less than correspondingly quicker because of the requirement for testing transformants.

In forest trees, the stage(s) in the life cycle at which introduced genes are expressed is potentially very significant. If such genes are only expressed during the first few years of the lifetime of the crop (e.g., with herbicide resistance) the potential seriousness of a disaster will be greatly reduced. Given the phenomenon of maturation ("physiological ageing") in most forest trees, there are definite prospects of the desired age-specific gene expression. Any consequent optimism must, however, be tempered by the consideration that, at least in *P. radiata* (Burdon & Miller 1992), maturation is a progressive and relatively gradual process rather than being abrupt.

What proportion of the total crop can be safely committed to the use of a particular introduced gene may depend on some additional factors. For instance, areas of crop that are marginal to the core requirements of heavily capitalised industrial plant can presumably be exposed to somewhat greater risks. Similarly, if the crop is only a small part of a production "portfolio", much higher levels of risk in growing it may be acceptable. Also relevant may be whether a transformation counters a known important hazard, such as a disease or pest.

If a satisfactory spread of the risks associated with genetic transformation cannot be achieved, and other avenues of risk mitigation are suspect, there should be a critical analysis of whether a breeding objective should actually be pursued and, if so, whether that can be done by conventional breeding. That need not, however, eliminate genetic transformation as a very powerful ancillary breeding tool, if it can be used to study developmental pathways and to help locate naturally occurring genes that meet the objective. Information acquired thus can be used either for exploiting such genes by classical breeding or for genetic transformation with minimal risk.

## Institutional and Legislative Issues for New Zealand

Prudent risk management would thus seem very important for operational use of genetic transformation. In New Zealand we have had a forestry sector with a structure that should

make that achievable, with the planting dominated by a few very large organisations. Recently, however, a high proportion, in some years over half, of the annual planting (including restocking) has come to be done by the "small players" which include small companies, syndicates, and even individuals (G.P.Horgan, pers. comm.). It will therefore be a challenge for the sector to observe the requisite discipline, even though forestry operations may form part of a risk spread for many of the small players.

While a disciplined approach to the use of genetic transformants may in theory be possible, their operational use may currently be precluded in New Zealand by the recent Hazardous Substances and New Organisms (HSNO) Act. This Act makes no provision for the approval of commercial release of genetically transformed organisms being made subject to certain risk management conditions being observed. The authority that administers the Act, the Environmental Risk Management Authority (ERMA), operates under a highly risk-averse brief, and the risks that it must address include economic risks right down to the level of communities. Its inability to impose conditions on a release of material for operational use constitutes an important gap in the provisions of the Act. While this gap is recognised, it could take some time to rectify the matter. The Act will need to be revised judiciously, because we do not want unduly restrictive provisions like the statutory prescriptions in parts of Europe for unrealistically high minimum numbers of clones for forest deployment.

## Concluding Remarks

What I have written in no way purports to be a definitive analysis of a topic that is likely to generate heated controversy. I have focused on a type of biological hazard that has received limited attention. There is a need to confirm and quantify the various biological phenomena that I have reviewed or postulated, and thence to elaborate the quantitative analysis. There is also a need to integrate the further analysis with risk analysis relating to classical clonal forestry (Roberds & Bishir 1996). Finally, I am preparing a review outlining the broader genetic aspects of risk management in New Zealand forestry (Burdon in prep.).

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