Environ. Biosafety Res. 9 (2010) 181–198
© ISBR, EDP Sciences, 2011
DOI: 10.1051/ebr/2011109

Available online at:
www.ebr-journal.org

Research report

Developing risk hypotheses and selecting species for assessing non-target impacts of GM trees with novel traits: The case of altered-lignin pine trees

Louise A. MALONE[1]*, Jacqui H. TODD[1], Elisabeth P. J. BURGESS[1], Christian WALTER[2], Armin WAGNER[2] and Barbara I.P. BARRATT[3]

[1] The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research), Mt Albert, Private Bag 92169, Auckland Mail Centre, Auckland 1142, New Zealand
[2] The New Zealand Forest Research Institute Limited (Scion), Te Papa Tipu Innovation Park, Private Bag 3020, Rotorua Mail Centre, Rotorua 3046, New Zealand
[3] AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand

A procedure is presented for developing environmental risk hypotheses associated with the deployment of forest trees genetically modified to have altered wood properties and for selecting non-target species to test these hypotheses. Altered-lignin *Pinus radiata* trees intended for use in New Zealand are used as a hypothetical case study to illustrate our approach. Firstly, environmental management goals (such as wood production, flood control or preservation of biodiversity) were identified and linked to the forest attributes they require. Necessary conditions for each attribute were listed and appropriate assessment endpoints for them developed. For example, biological control of pests may be one condition necessary for a forest to have healthy trees, and the diversity and abundance of natural enemy species in the forest could be an appropriate assessment endpoint for measuring this condition. A conceptual model describing the relationships between an altered-lignin GM pine tree and potentially affected invertebrates and micro-organisms in a plantation forest was used to develop a set of risk hypotheses describing how the GM trees might affect each assessment endpoint. Because purified lignin does not represent the properties it imparts to wood, maximum hazard dose tests with non-target organisms, as are used to inform toxin risk assessment, cannot be conducted. Alternative experiments, based on current knowledge of the responses of organisms to lignin, must be designed. A screening method was adapted and applied to a database of invertebrate species known to inhabit New Zealand pine forests to identify and prioritize non-target invertebrate species that could be used as experimental subjects for examining these hypotheses. The screening model and its application are presented, along with a set of recommendations for pre-release tests with GM pines and potentially affected invertebrates and micro-organisms.

Keywords: genetically modified trees / altered lignin / non-target invertebrates /ecological risk assessment

INTRODUCTION

The potential for genetic modification (GM) to improve plantation forestry, by reducing the need for pesticides, increasing yields, improving wood quality and reducing processing costs (including environmental costs), has long been recognised (Valenzuela et al., 2006). More than 700 field tests with GM trees have been conducted worldwide, and environmental impact studies have not identified negative environmental impacts (Walter et al., 2010). Even so, at present GM trees are a commercial reality only in China, with about 1.4 million Bt poplars planted since 2002 (Huang et al., 2007), and Hawaii, where genetically modified papaya plants resistant to the papaya ringspot virus have sustained the papaya industry since 2001 (Ferreira et al., 2002).

However, the recent granting of three separate approvals for GM tree field trials for testing traits of potential commercial interest, one in the United States (USDA, 2010), one in Belgium (Anon, 2005a) and one in

* Corresponding author: louise.malone@plantandfood.co.nz

New Zealand (ERMANZ, 2010), suggests that this situation is about to change. In the United States, field trial approval was granted in late 2009 by the United States Department of Agriculture (USDA) for *Eucalyptus grandis* Hill ex Maid x *Eucalyptus urophylla* S.T. Blake hybrids modified by the insertion of genes conferring cold-tolerance, altered fertility and altered lignin biosynthesis (USDA, 2010). In Belgium, a field trial of *Populus tremula* L. x *Populus alba* L. (*Populus* x *canescens*) (grey poplar) modified to have decreased activity of lignin biosynthesis enzymes and increased bio-ethanol yields gained final approval only after the reversal of an earlier negative decision by Federal Ministers (Anon, 2005a). In New Zealand, an approval to field-test *Pinus radiata* D. Don (Monterey pine) trees genetically modified to alter biomass acquisition, biomass utilization, wood density, stability, reproductive development or to be herbicide tolerant was granted by the regulator (ERMANZ, 2010).

The modification of lignin biosynthesis is an attractive target for genetic engineers of forest trees (Vanholme et al., 2008). Lignin is a major component of plant cell walls and wood properties are strongly influenced by the ratio of lignin to cellulose and the relative amounts of different types of lignin in the tree. By modifying lignin biosynthesis, trees with profoundly different phenotypes can be produced and the trees' value for producing timber, pulp, paper or biofuel can be significantly increased (Boerjan et al., 2003).

GM trees are likely to pose new challenges for risk assessors and biosafety researchers. As Wilkinson and Tepfer (2009) recently pointed out, current environmental risk assessment practice for GM plants has arisen from extensive experience with four arable crops (maize, cotton, soybeans and oilseed rape) and two types of relatively simple, "foreign protein-mediated" traits (Bt-based insect resistance and herbicide tolerance). The GM trees being field-tested at present, however, differ in two important ways: they are long-lived plants and their physiology has been deliberately changed by their modification.

The US, Belgian and New Zealand field trials include trees with lignin modifications. The dossiers of information on their potential environmental impacts submitted to regulators included consideration of gene flow, weediness, horizontal gene transfer, fire risks, soil hydrology effects, allelopathy, cryptococcal disease, risks to endangered or threatened species (mostly vertebrates) and impacts on non-target organisms (Anon, 2007; Anon, 2008; ERMANZ, 2010; USDA, 2010). The US and Belgian dossiers noted that the trees were not intended to affect any target organisms and that non-target effects were also unlikely. Both indicated that they would be monitoring for pest insects and diseases and would notify the authorities of any unexpected effects. The Belgian applicant was instructed by the regulators to conduct field surveys to check for impacts on insect and soil microbial biodiversity (Anon, 2008). The New Zealand application indicated that impacts on insects and micro-organisms would be assessed during the trial (ERMANZ, 2010).

Plantations of exotic tree species represent the second largest use of agricultural land in New Zealand (Anon, 2005b) and New Zealand has significant scientific capability in forest biotechnology. The eucalyptus hybrids for the US field trials were transformed in New Zealand (USDA, 2010) and GM conifers carrying transgenes for herbicide tolerance and insect resistance, as well as marker and resistance genes, have been produced in this country (Bishop-Hurley et al., 2001; Find et al., 2005; Grace et al., 2005). Field trials in New Zealand have assessed the environmental safety of GM pines carrying transgenes to alter the development of flowers and cones and antibiotic resistance markers and confirmed no negative impacts on invertebrates and soil microorganisms (Lottmann et al., 2010; Schnitzler et al., 2010). However, commercial development of GM trees for planting in New Zealand has not proceeded rapidly. This has afforded us the opportunity to address environmental risk assessment issues early in the development process, although there are drawbacks to this as well. Trees take a long time to produce wood and purified lignin cannot be extracted in authentic form from wood. Unlike Bt toxins, which can be purified and for which there is an extensive body of knowledge of non-target effects to guide risk hypothesis formulation, we have only incomplete knowledge of the impacts of different types of lignin on other organisms and no standardized laboratory bioassays with which to conduct preliminary investigations. We selected lignin-altered *P. radiata* as one of several New Zealand case studies involving "metabolically modified" long-lived GM plants (see also Barratt et al., in press) in order to gain experience in developing logical, comprehensive and cost-efficient approaches to GM environmental risk assessment and to learn how much of this process could be considered generic.

Here we describe risk hypothesis formulation for a long-lived, physiologically altered GM crop and a technique for selecting non-targets potentially at risk in the absence of data from Tier 1 toxicity tests. Tier 1 tests are conducted for Bt and other toxins by feeding purified toxin at a "maximum hazard dose" that is several times the expected environmental concentration of the toxin (Romeis et al., 2011). Because lignin cannot be purified in natural form, such tests cannot be conducted with this

https://doi.org/10.1051/ebr/2011109 Published online by Cambridge University Press

compound. Our aim was to provide a method that could be used to focus resources on tests yielding the most informative results for regulators assessing environmental risk and making decisions about the cultivation of GM trees.

We used a "top-down" approach, beginning with identification of environmental management goals relevant to New Zealand pine plantations, the forest attributes needed to meet these goals and the necessary conditions for a forest to have these attributes. The United States Environmental Protection Agency (USEPA) described this approach to environmental risk assessment for all kinds of potential stressors in 1998 (USEPA, 1998). Another "top-down" approach, where "ecological function" is used as a primary criterion for the selection of non-target species for tests with GM plants was first suggested by Andow and Hilbeck (2004) and was subsequently explored further in case studies with Bt maize in Kenya (Hilbeck and Andow, 2004), and Bt cotton in Brazil (Hilbeck et al., 2006) and Vietnam (Andow et al., 2008). A recent guidance document on GM plant biosafety tests issued by the European Food Safety Authority (EFSA) also recommends the selection of non-target species to represent each ecological functional group in the receiving environment (EFSA, 2010a; b).

For New Zealand to meet its environmental management goals in relation to pine plantations, we identified the conditions that would be necessary for this and assessment endpoints that could be used to measure these. We then built a conceptual model to estimate the likely impact of lignin alteration on endpoints involving invertebrates and micro-organisms, the most diverse taxa in these plantations. A set of risk hypotheses describing predicted relationships between altered-lignin GM pine and potentially affected organisms was then developed. Lists of species, ranked in order of suitability as potential test subjects, were then produced by applying a screening model (adapted from Todd et al. (2008)) to a database of 251 invertebrates known to occur in New Zealand pine plantations. Some recommendations for experiments to measure the potential non-target impacts of altered-lignin GM pines are presented.

Reasons for initiating an environmental risk assessment of lignin-altered pines

The purpose of any lignin modifications proposed for *P. radiata* in New Zealand would be to improve the processing characteristics and/or the quality of the wood produced. Further, the calorific value of wood and its biochemical composition can be modified to enhance its suitability for bioenergy and biomaterials applications. An environmental risk assessment would be required by law, not because these trees have been demonstrated to affect the environment negatively, but because they will be genetically modified, and hence considered "new organisms" (Hazardous Substances and New Organisms (HSNO) Act 1996). Thus the process of selecting assessment endpoints in this case is "value-initiated" (to use the terminology employed by the USEPA (1998)) and the process will be driven by goals for the ecological values of concern. These values may be better understood by exploring the desired environmental attributes of pine plantations in New Zealand and examining the New Zealand regulations governing the planting of GM crops.

From a commercial perspective, *P. radiata* trees are grown for wood and pulp production in New Zealand on an area of about 1.7 million hectares (Dennis et al., 2005). This species grows faster under New Zealand conditions than it does in its native California and pests are largely absent, resulting in a 28-year rotational cycle with little need for pest management (or pesticide use). Efficient production relies on the preservation of ecological processes such as decomposition, nutrient cycling, and suppression of herbivorous arthropods by natural enemy species. Plantation forest ecosystems also contribute to more general environmental goals by providing habitats for native species (Colbourne and Kleinpaste, 1983; Pawson et al., 2010), protection of biodiversity, low greenhouse gas emissions, carbon sequestration, erosion control, providing clean streams and water catchment areas, and reducing flood risks. New Zealand's only native mammals are two species of rare bats; introduced mammals found in pine plantations, such as deer, rabbits and possums, are considered to be pests and/or game. Plantations also contribute to the social goals of employment and provision of public places for recreational pursuits such as cycling, hiking, horse riding, dog walking, bird watching, fishing and hunting.

In New Zealand, the HSNO Act is administered by the Environmental Risk Management Authority (ERMA New Zealand) – recently renamed the Environmental Protection Agency –, which evaluates and reviews environmental risk assessments and determines whether, and under what conditions, GM plants are deployed in this country. This legislation articulates some ecological and social goals that might also be used to define desirable environmental attributes of *P. radiata* pine forests: "safeguarding of the life-supporting capacity of air, water, soil, and ecosystems", "the sustainability of all native and valued introduced flora and fauna", "the intrinsic value of ecosystems", and "the relationship of Māori (New Zealand's indigenous people) and their culture and

https://doi.org/10.1051/ebr/2011109 Published online by Cambridge University Press

traditions with their ancestral lands, water, sites, waahi tapu (sacred sites), valued flora and fauna, and other taonga (treasures)". "New Zealand's international obligations" must also be taken in account (New Zealand has ratified the Convention on Biological Diversity). Section 36 "minimum standards" in the HSNO Act stipulate that applications to release GM organisms (GMOs) must be declined if it can be shown that the GMO will displace native species, cause deterioration of natural habitats, or significantly adversely affect "New Zealand's inherent genetic diversity".

Assessment endpoints

Relationships between desirable forest attributes (*e.g.*, healthy trees, clean streams) and environmental management goals (*e.g.*, preservation of biodiversity, carbon sequestration) for *P. radiata* plantations in New Zealand are shown in Figure 1. Table 1 identifies some of the conditions and ecological processes required to maintain those attributes (*e.g.*, efficient nutrient cycling) and suggests some examples of assessment endpoints that could be used to measure and manage risks from stressors such as GM pines. For the rest of this paper, we will focus only on endpoints involving invertebrates or microbes, as these are by far the most diverse taxa in New Zealand pine plantations (Tab. 1).

Possible impacts of altered-lignin GM pines on invertebrate and microbial assessment endpoints

Lignin is the third-most abundant biopolymer, after cellulose and hemicellulose of terrestrial plant cell walls (Uprichard, 1991). It is the component of wood that acts as the 'glue' to combine hemicellulose polymers together as a composite with cellulose crystallites in the cell wall. The distribution of lignin affects wood properties such as stability, and also affects the pulpability of wood harvested from trees. The most abundant lignin monomers (monolignols) in tree species are *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. These monolignols contribute to the formation of different lignin types called *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) lignin, respectively. Lignin in gymnosperms, such as *P. radiata*, generally consists almost entirely of G with small quantities of H (Nanayakkara et al., 2009), whereas dicotyledonic angiosperms usually have lignin composed of a mixture of G and S. Genetic modifications to alter lignin biosynthesis in trees are intended either to alter the total concentration of all lignin types or to alter the ratios of the different types of lignin to one another. A number of different strategies have been developed for modifying lignin content and/or composition; most involve suppression or overexpression of genes in the

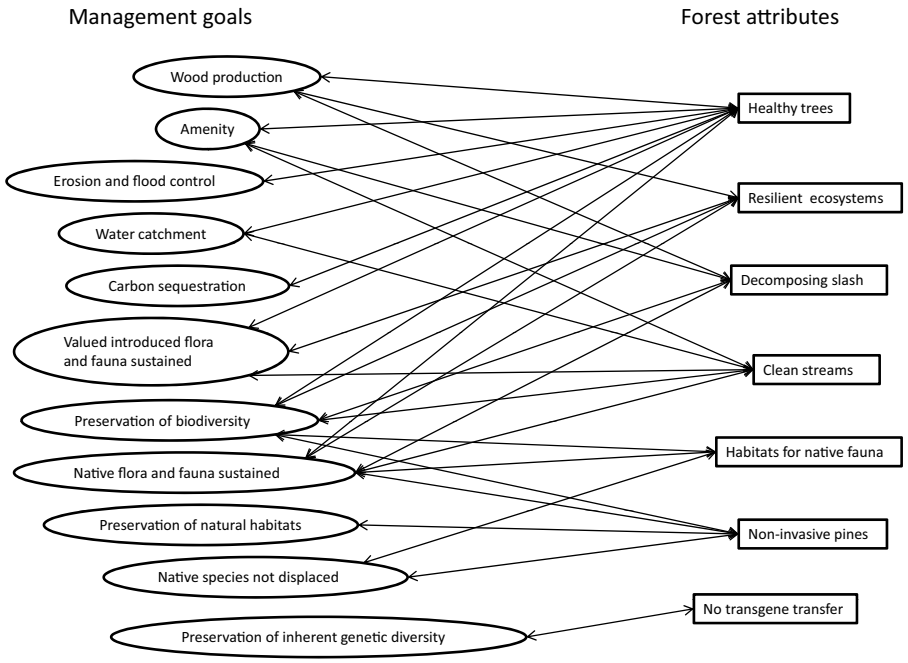

Figure 1. Relationships between environmental management goals New Zealand *Pinus radiata* plantation forests and their attributes.

https://doi.org/10.1051/ebr/2011109 Published online by Cambridge University Press

Table 1. Desired environmental attributes of *Pinus radiata* D. Don (pine) plantations in New Zealand, with some conditions necessary for their maintenance, examples of assessment endpoints that could be used as measures of these attributes, and the potential for a lignin modification to have any conceivable impact on that endpoint.

Required attributes	Necessary conditions	Assessment endpoints	Potential for lignin modification to affect endpoint
Healthy pine trees	Efficient nutrient cycling	Pine tree growth rate*	None
	Biological control of pests and diseases	Diversity and abundance of natural enemy species (*e.g.*, parasitoids known to prey on defoliating insects)[1]	Indirect
		Occurrence of tree diseases[1]	Direct
	Border biosecurity – detection, eradication or control of new pests and diseases	Population size and reproductive success of pest species (*e.g.*, arthropod[1] and mammalian defoliators)	Direct
	Beneficial mycorrhizal associations	Abundance of beneficial mycorrhizae[1]	Direct
	No fires	N/A	N/A
	No floods	N/A	N/A
	No windthrow	N/A	N/A
	Efficient water use by trees	Pine tree growth rate*	None
Resilient ecosystems	Multitrophic, complex food webs remain intact	Diversity and abundance of species representing different trophic levels/functional groups[1]	Direct and indirect
		Diversity and abundance of understorey plant species	None (unless GM trees compete better than non-GM)
		Diversity of birds and reptiles	Indirect
		Trout, eel, koura (crayfish[1]) counts/catch figures	Indirect
	Pockets of native forest retained as refugia for native species	Land area of native forests adjoining pine plantations	None
Decomposition of slash	Diverse and abundant wood-decomposing biota	Diversity and abundance of wood-decomposing species[1]	Direct
		Rate of decomposition of felled logs and slash left in forest[1]	Direct
Clean streams	Biological control of pests (and diseases) (no pesticides)	Abundance and diversity of aquatic species, including microbes	Direct
		Populations of native aquatic invertebrates[1] (koura) and vertebrates (eels)	Indirect
		Trout population size	Indirect
	Efficient nutrient cycling (no fertilisers)	Physico-chemical measures of water quality	Indirect
Habitats and food for native arthropods, birds, reptiles and bats (in pine forest)	Diverse and abundant refugia and niches for arthropod communities	Abundance and diversity of native arthropod species[1]	Direct or indirect
	Diverse understorey plants	Abundance and survival of native bird, bat or reptile species in pine forests	Indirect
	Healthy trees	Nest sites for native bird species	None (assuming no change in numbers or sizes of tree-holes)
		Nest sites for native bats	None
	Alternative tree species (eucalyptus) adjacent to pine forests (mixed stands at landscape level)	Food sources, shelters and egg-laying sites for native arthropods[1]	Direct (for herbivores, omnivores, shelter- builders, egg-layers)
Pines do not have enhanced invasiveness	GM pines do not have enhanced dispersal ability (seeds) (*P. radiata* has no wild or weedy relatives in NZ)	*P. radiata* seed production, mobility and viability	None
		Distribution of "wilding pines"	None
		Presence of (unplanted) pine trees in adjacent natural habitats	None
	Established GM pines do not compete better than non-GM	Pine tree growth rate*	Direct
GM pines do not transfer genes to other organisms	Pines do not cross with other plant species	Presence of transgenes in non-planted trees	None
	No abnormal horizontal gene transfer	Presence of transgenes in soil bacteria[1]	None

* Production standards for commercial selection of the trees will eliminate any GM lines that do not grow as well as non-GM trees, but trees with enhanced growth may be selected and be more competitive than non-GM pine trees.
[1]Assessment endpoints that may involve invertebrates or micro-organisms.
N/A: Not applicable, *i.e.* affected by wind, rain or fire, not biotic factors.

https://doi.org/10.1051/ebr/2011109 Published online by Cambridge University Press

lignin biosynthesis pathway (*e.g.*, Huntley et al. 2003; Wagner et al., 2009; 2007). More recent strategies include the introduction of new biosynthetic pathways (*e.g.*, Grabber et al., 2010). Although most lignin occurs in the woody tissues, such as the trunk, branches and roots, some can also be found in leaf tissue. For example, untransformed fresh *P. radiata* needles were found to contain 22% (w:w) lignin (Parfitt and Newman, 2000). Any alteration to the type or concentration of lignin in a GM tree could reasonably be expected to alter the toughness of these lignin-bearing tissues. This might in turn affect the ability of microbes and other organisms to decompose dead wood and needles, and to form mutualistic or parasitic associations with live trees, and it could affect the ability of herbivores to utilize the trees as sources of food, nest-building materials, or egg-laying sites. Any risk assessment also needs to take into account the large natural variation of lignin content in pines and any effects on non-target organisms would need to be evaluated in this context.

Until recently, it was widely accepted that most insects were unable to digest lignin (Prins and Kreulen, 1991). However, examination of the chemical structure of lignin in frass samples from two wood-feeding insects, a termite that eats dead wood and a cerambycid beetle that eats live wood, has provided evidence of lignin degradation in the insect gut, probably involving communities of unidentified symbiotic micro-organisms (Geib et al., 2008).

Plant tissue toughness can significantly affect the ability of plant-chewing insects to obtain nutrition (Clissold et al., 2009). Members of different insect feeding guilds will respond differently to plant toughness. Peeters et al. (2007) noted that the density of leaf-chewing species was affected by lignin content of different plants in an ecosystem, but sucking insects were not so affected. Root-chewing wireworms took longer to chew through roots of altered-lignin GM tobacco plants with enhanced fracture toughness (Johnson et al., 2010). In another laboratory study, lignin-altered GM birch leaves had no significant impact on insect performance (Tiimonen et al., 2005). Similarly, a survey of insects and insect feeding damage on altered-lignin GM poplars in the field revealed no differences that could be attributed to the modification (Pilate et al., 2002). Decomposition of leaf litter from altered lignin GM poplars was reduced compared with non-GM poplar leaf litter, but aquatic insect communities on litterbags placed in streams did not differ with plant type (Axelsson et al., 2010). The impacts on insects of a range of types of lignin modification have not yet been assessed and neither have those of modified gymnosperms.

Lignin plays an important but not yet fully understood role in interactions between trees and microbes. Plantation forest health depends on communities of fungi and bacteria which can act as beneficial mutualists (ectomycorrhizae), saprophytes or parasites.

As lignin is a major component of dead wood and pine needles, one might expect alterations in lignin composition to affect decomposition rates. In some fungi, the secretion of ligninolytic enzymes (laccases) is stimulated by the presence of lignin (Punelli et al., 2009) and alterations in the types of lignin might affect these responses. Wood-decaying fungi vary in their ability to digest lignin; for example, most "white rot" fungi on *P. radiata* wood secrete ligninolytic enzymes, but most "brown rot" fungi are less able to digest lignin (Ferraz et al., 2001). Altering the type of lignin in a tree may affect the composition of decomposer communities, although the net effect on decomposition may not be significant. Studies to date with altered-lignin GM plants indicate that changes in decomposition rates could be variable and greatly influenced by soil type, presence of earthworms, and other environmental factors (Bradley et al., 2007; Hancock et al., 2008; Henault et al., 2006).

Plant cell walls can act as physical barriers to attack by plant pathogens. As lignin contributes to cell wall toughness, its alteration may affect tree responses to pathogens. Recent studies show that lignin biosynthesis pathways are involved in plant defence responses to pathogenic micro-organisms. For example, trees inoculated with pitch pine canker fungus produced heavy deposits of lignin at the wound site (Kim et al., 2009). *Arabidopsis thaliana* L. (Hehn.) plants challenged with *Pseudomonas syringae* pv. *tomato* (Okabe) Young et al. show altered gene expression for enzymes in the lignin biosynthesis pathway (Tronchet et al., 2010), and *P. radiata* cells in culture responded to challenge with *Dothistroma pini* Hulbary fungus by producing not only an oxidative burst, but also higher amounts of an enzyme involved in lignin biosynthesis (Hotter, 1997), reflecting a change in lignin content and composition in pine needles infected with *D. pini* or after dothistromin toxin injection (Franich et al., 1986). Recently, GM poplars have been used to demonstrate the role of a transcription factor in this species' response to challenge with rust fungus, a reaction which includes changes in lignin deposition (Levee et al., 2009). We are not aware of any studies of defence responses in altered-lignin GM trees, but we can speculate that some impacts might be possible.

https://doi.org/10.1051/ebr/2011109 Published online by Cambridge University Press

Symbiotic ectomycorrhizae play an important role in pine tree ecosystems, improving tree growth by facilitating access to nutrients. It is very unlikely that alterations in lignin composition of tree roots could affect these beneficial associations, since these fungi do not penetrate the tree's cell walls. The few studies to date with lignin-modified GM birch and its ectomycorrhizal fungus *Paxillus involutus* (Batsch) Fr. have produced only limited evidence of impacts (Seppanen et al., 2007; Sutela et al., 2009; Tiimonen et al., 2008).

Assessment endpoints involving invertebrates or micro-organisms that could conceivably be affected by lignin modification are identified in Table 1.

Conceptual models and risk hypotheses concerning invertebrates and micro-organisms

Figure 2 depicts a conceptual model illustrating possible relationships between altered-lignin GM pine and invertebrates and micro-organisms potentially affected by lignin modification (see Tab. 1). Pines with altered lignin content or composition are likely to have leaves, roots and wood (both live and dead) with altered toughness (harder or softer) compared with non-GM pines. These changes could affect any organisms that feed on, burrow into, or lay eggs in any part of the pine tree (living or dead), including saprophytic or pathogenic microbes associated with the tree. Any organisms that prey upon others potentially affected by the GM pine could also be affected indirectly (tri-trophic interactions or population effects). Furthermore, there are a number of species' characteristics that could serve to mitigate these potential impacts. For example, species that can feed on alternative plants and are mobile enough to do so may not be as affected as those with more restricted food ranges or mobility. At a population level, mobile species with broader geographical or ecological ranges might recover from an impact more quickly than species with more limited distributions.

Table 2 shows a set of risk hypotheses derived from consideration of these potential changes in the pine trees and some of the entity-tree relationships depicted in Fig. 2.

With our current state of knowledge, we cannot yet reasonably assess the likelihood or magnitude of these risks, except perhaps to suggest, based on previous studies with other modified pines (Barraclough et al., 2009), that effects on natural enemies of species directly exposed to lignin-bearing plant parts are likely to be less than any effects on their prey. The risk hypotheses suggest a number of obvious experimental hypotheses that could serve as the basis for biosafety tests, for example, that the growth and survival of a wood-boring arthropod fed with altered-lignin GM pine wood would be different from that of one fed with control pine wood. There are additional experimental hypotheses that could also be investigated to provide a better basis for risk assessment: 1) that species that can feed on, lay eggs in, or build shelters using plants

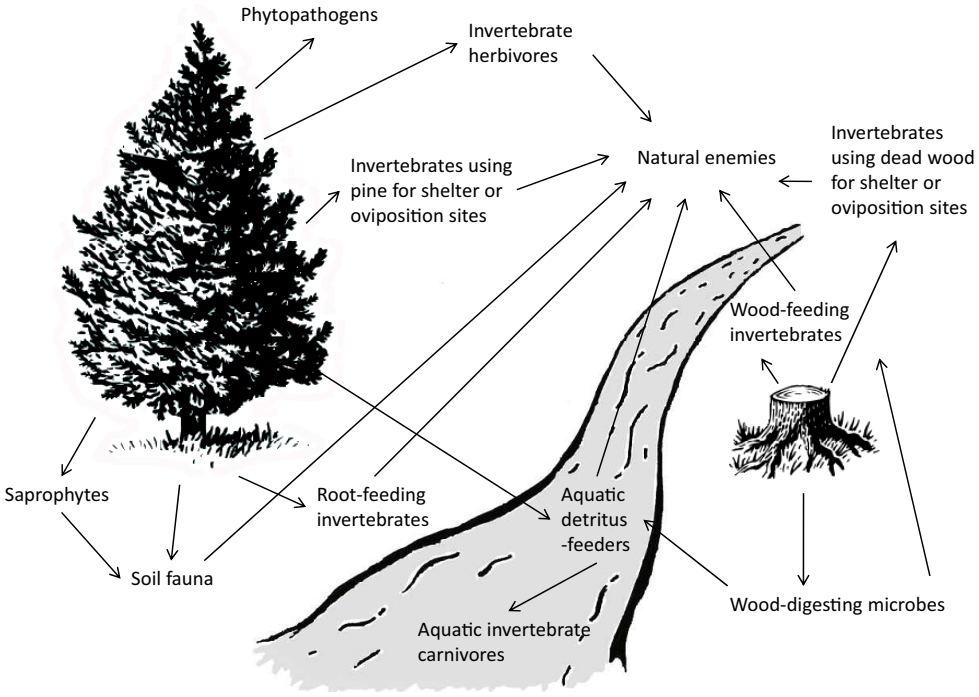

Figure 2. A conceptual model showing relationships between *Pinus radiata* and micro-organisms and invertebrates that might be affected by a genetic modification to alter the lignin characteristics of the tree.

https://doi.org/10.1051/ebr/2011109 Published online by Cambridge University Press

Table 2. A set of risk hypotheses describing predicted relationships between altered-lignin GM pine and the potentially affected invertebrates or micro-organisms identified in Table 1 and Fig. 1*.

Entities	Risk hypotheses
Herbivores and omnivores: leaf-feeders, root-feeders, live- and dead-wood feeders, sap suckers	Growth and survival of live-wood-chewers will be affected Growth and survival of root-chewers will be affected Growth and survival of leaf-chewers will be affected
Detritivores (aquatic or soil-inhabiting)	Growth and survival of detritivores feeding on dead pine needles or dead wood will be affected
Invertebrates that use pine trees and their parts for shelter: wood-borers, leaf-folders, species that use parts of needles to build shelters or camouflage structures (*e.g.*, bag moths)	Organisms that use pine foliage or wood to build shelters ("shelter-builders") will be affected
Invertebrates that lay their eggs inside pine needles or in live or dead pine wood	Organisms that lay their eggs inside pine foliage or wood ("egg-layers") will be affected
Natural enemies (parasitoids, predators) of any affected organisms (feeders, shelter-builders or egg-layers)	Growth and survival of organisms that prey on or parasitise others that may be affected by GM pine will also be affected Growth and survival of omnivores whose diets include pine-feeding organisms will be affected
Saprophytic microbes	Growth and survival of saprophytic microbes will be affected The rate of decomposition of wood and foliage from GM pines will be altered
Pathogenic microbes	Establishment, growth and survival of pine pathogens will be affected

*Because we do not yet know if the GM pine will be tougher or softer than non-GM pine, we can hypothesise only that some species will be affected, not whether they will be harmed or will benefit from the change. Potentially a benefit to an individual species could represent a risk to the ecosystem, for example a non-pest herbivorous species might attain pest status through population growth if the GM pine is a better food source than non-GM pine.

other than pine are less likely to be affected (when given a choice of plants) than those that specialise on pine, 2) that species with limited mobility that feed on, lay eggs in, or build shelters using altered-lignin GM pine are more affected in the field than those with greater mobility, 3) that sucking herbivores are not as affected as chewing herbivores, and 4) that xylem-feeders are more affected than phloem-feeders.

Thus there is a manageable number of experiments that could be carried out to help with risk assessment of altered-lignin GM pine. The next step in our process is to decide on the best species to use as test subjects in these experiments. There are hundreds of invertebrate species and unknown numbers of microbes inhabiting New Zealand pine plantations, leaving us with an almost bewildering array of candidates for experiments. Several criteria have been proposed for selecting non-target species for tests with insect-resistant GM plants. For these types of GM plants, there is agreement that potential susceptibility and exposure to the expressed toxins are key criteria (Andow, 2011; Raybould et al. 2011; Romeis et al., 2011), but views on the value of other criteria differ. Romeis et al. (2011) argue that amenability to testing and availability of the species are important and Raybould et al. (2011) stress the sensitivity of the chosen species and their ability to act as surrogates for other species in the receiving environment. Andow (2011) and EFSA's Panel on GMOs (EFSA, 2010a; b) stress the ability of the test species to represent the ecological functional group to which it belongs; both also argue that this approach can be applied to a variety of GM plants with a variety of traits, not just resistance to pest insects. The USEPA's criteria for selecting assessment endpoints for ecological risk assessments combine both viewpoints and so may be useful for our GM pine case study, namely: ecological relevance, susceptibility (and exposure) to the stressor, and relevance to management goals. Availability of specimens and laboratory handling techniques will also be important if resources are to be used wisely, although this alone would not be an adequate criterion for selection.

RESULTS

A model for prioritising invertebrate species as test subjects

A screening model that automatically applies a set of prioritisation criteria to an extensive list of test subject candidates could help to ensure that tests yielding information of most use to risk assessors are performed first.

https://doi.org/10.1051/ebr/2011109 Published online by Cambridge University Press

Table 3. A list of ten invertebrate species ranked in order of suitability as test subjects for assessing the impacts of altered-lignin GM pine in New Zealand, based on the likely impact of lignin alterations on them *via* their feeding, shelter-building and/or egg-laying habits, and their potential to avoid or mitigate this impact (F) and the anthropocentric value of each species (V) (see Methods). Each species position when ranked according to F alone is also given, as "RankF".

Rank FV	Species	Family	Order	Feeds on	Lays eggs in	Builds shelters	F	RankF	F + V/10
1	*Euophryum rufum* (Broun)	Curcul.	Col.	Dead wood	Dead wood	Tunnels in wood	34.1	1	36.6
2	*Prionoplus reticularis* White	Ceramb.	Col.	Dead wood	Dead wood	Tunnels in wood	32.1	6	35.1
3	*Pycnomerus* Erichson sp.	Zoph.	Col.	Dead wood	Unknown	Under bark	32.2	4	34.7
4	*Torostoma apicale* Broun	Curcul.	Col.	Dead wood	Unknown	Tunnels in wood	32.5	3	34.5
5	*Microcryptorhynchus kronei* (Kirsch)	Curcul.	Col.	Dead or dying wood	Dead wood	Tunnels in wood	33.0	2	34.5
6	*Mitrastethus baridioides* Redtenbacher	Curcul.	Col.	Wet rotting wood	Unknown	Tunnels in wood	32.1	5	34.1
7	*Kalotermes brouni* Frogatt	Kaloter.	Iso.	Dry dead wood	Dry dead wood	Dry dead wood	30.9	10	33.4
8	*Calliprason pallidum* (Pascoe)	Ceramb.	Col.	Dead or dying wood	Bark crevices	Tunnels in wood	31.2	7	32.9
9	*Pachycotes peregrines* (Chapius)	Curcul.	Col.	Dead wood	Freshly felled logs	Tunnels in wood	30.5	13	32.5
10	*Leanobium flavomaculatum* Espanol	Anob.	Col.	Dead wood	Bark crevices	Tunnels in wood	30.7	11	32.4

Abbreviations: Anob. = Anobiidae; Ceramb. = Cerambycidae; Col. = Coleoptera; Curcul. = Curculionidae; Iso. = Isoptera; Kaloter. = Kalotermitidae; Zoph. = Zopheridae.
A full list of species is available from the corresponding author.

Table 3 presents part of a list of invertebrate species and their priority rankings ("RankF") based on their likelihood of being affected and thus their potential as suitable subjects for testing risk hypotheses involving invertebrates for altered-lignin GM pine under New Zealand conditions. The list was produced by modifying the PRONTI (Priority Ranking of Non-Target Invertebrates) model (Todd et al., 2008) and applying it to a database containing biological and ecological information on over 1300 New Zealand invertebrate species found in productive ecosystems (hereafter referred to as the Eco Invertebase).

For each species known to occur in pine forests and recorded in the database, a "species score" (F) was calculated using a series of scores that had been assigned to the answers to questions relevant to the impacts of altered lignin pines. The assignment of scores to answers was a subjective process. High scores (usually up to 10) were given to answers that would suggest the potential for a species to be highly exposed to or affected by an alteration in the lignin composition of pine trees. Conversely, low scores were assigned to answers that would indicate a minimal effect of lignin modification on the species characteristic in question (*e.g.*, laying eggs on the surface of foliage). Where there was no information available for a particular species with respect to a characteristic, an entry of "unknown" was made; these were awarded nominal scores in the middle of the available range (usually 5). Any null entries (*e.g.,* for parts of pine trees eaten by a strictly carnivorous species) received a score of zero, unless otherwise stated. The calculation of F involved an assessment of the impacts (I) of the GM pines on each invertebrate species and also their potential ability to avoid or mitigate those impacts (M), such that $F = I - (M/10)$. Species characteristics of anthropocentric value, such as rarity, origin (endemic, native or exotic) and significance to indigenous people, were used to produce an additional score, V, for each species. V and F could be combined to produce a new species score, FV, for ranking in situations where regulators consider it appropriate for anthropocentric values to inform the risk assessment. Methods for calculating F, I, M and V are described in detail in the Methods section below.

The modified model assigned "impact scores" (I) to species based on their occurrence in pine forests or on pine trees, their feeding, egg-laying and shelter-building habits, and how those characteristics might predispose them to being affected by altered-lignin GM pines. This resulted in groups of species with similar I scores, forming a series of "steps" when plotted in descending order (Fig. 3). Dead-wood-feeders (weevils, termites, ironclad and longhorn beetles) that tunnel and lay eggs in pine wood ranked most highly. Species from similar families, with similar habits but greater host plant ranges, formed a second group. The third group comprised both wood-feeders and leafroller (tortricid) caterpillars that chew on pine foliage and use pine needles to build shelters. Leaf-chewing Lepidoptera (such as geometrids) that feed on pine but do not use the needles for shelters were ranked lower, along with Hemiptera that feed on sap, such as cicadas, scale insects,

https://doi.org/10.1051/ebr/2011109 Published online by Cambridge University Press

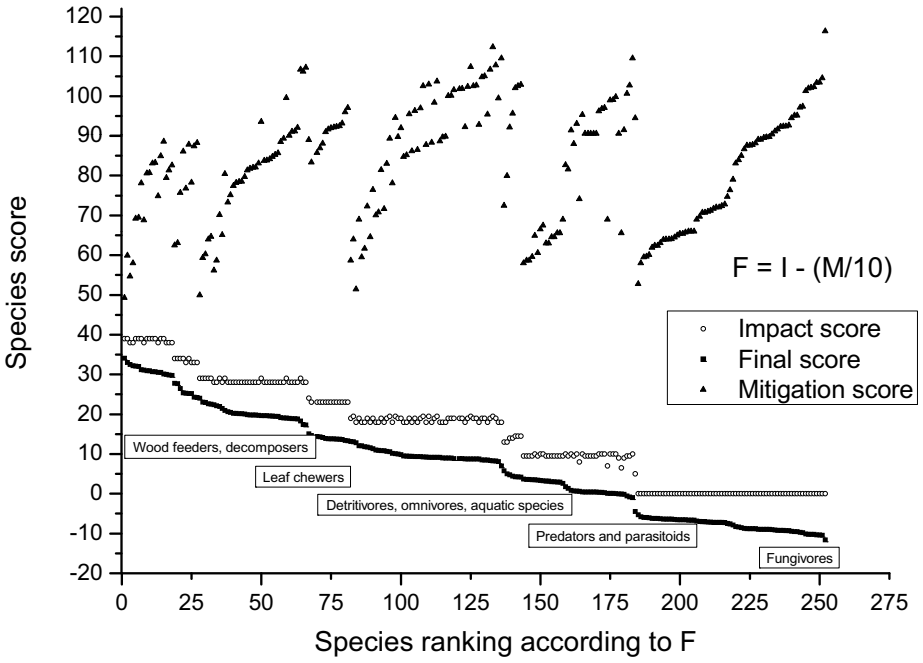

Figure 3. Graphical representation of invertebrate species found in New Zealand *Pinus radiata* forests ranked according to the likelihood of their being affected by a genetic modification to alter the lignin characteristics of the trees (I), their ability to mitigate this impact (M), and a combination of the two (F).

and woolly aphids. The next two groups of species included aquatic detritus feeders, with those that may use pine detritus to build shelters ranked above those that have greater food plant choices and do not build shelters. Aquatic omnivores ranked next, along with terrestrial predators known to feed on pine-feeding herbivores, followed by generalist predators, parasitoids, and other species that do not rely on pine, such as fungivores.

To separate the species on the list further and to facilitate the selection of a few to represent those potentially affected by altering the lignin composition of pine, we then calculated a "mitigation factor" (M) for each species. This was based on characteristics that might allow a species to avoid, mitigate or be resilient to the impacts of the GM pines. Mobility, dispersal distances, food species, geographical ranges, and reproductive capacity were included in this calculation, even though there were more "unknowns" for these attributes for many species in the database than was the case for the attributes used to calculate I (Fig. 4). Because of this uncertainty, M was divided by ten (a factor chosen to produce a noticeable gradient between species with the same I value, without generally altering the rankings) before being subtracted from I to produce a "final score" (F) for each species, *i.e.*

$$F = I - (M/10)$$

These final scores have no biological meaning; they simply serve as a basis for ranking species relative to one another according to their potential susceptibility to hypothesised risks from the GM pines. The different groups of species identified when ranked according to I (described above) appeared in a similar order when F was used to rank them, but the "stepped" appearance of the line when score is plotted against rank was "smoothed" by the inclusion of M in the calculation of each species score (Fig. 3). Thus a cerambycid beetle species such as *Prionoplus reticularis* White (I = 39, F = 32.1) is "promoted" up the list relative to the termite *Kalotermes banksiae* Hill (I = 39, F = 30.7) because the termite has a greater reproductive capacity, has been recorded in wood from a greater number of tree species, and has been collected from eucalyptus plantations as well as pine plantations and native forests, suggesting this species could be more resilient to the impacts of altering pine lignin than the cerambycid.

Applying anthropocentric values

Further separation of species was achieved by applying additional selection criteria, as in Todd et al. (2008). New Zealand environmental protection legislation explicitly mentions preservation of native species and valued exotic species, and species of significance to indigenous people, reflecting some unique aspects of New Zealand's biota, agriculture, and culture. New Zealand's invertebrate biota has extremely high endemism; for example, it has been estimated that 90% of coleopteran species are endemic (Watt, 1982). In contrast, the food and fibre crops of New Zealand are virtually all exotic species. The rights of New Zealand's indigenous people (Māori), including

https://doi.org/10.1051/ebr/2011109 Published online by Cambridge University Press

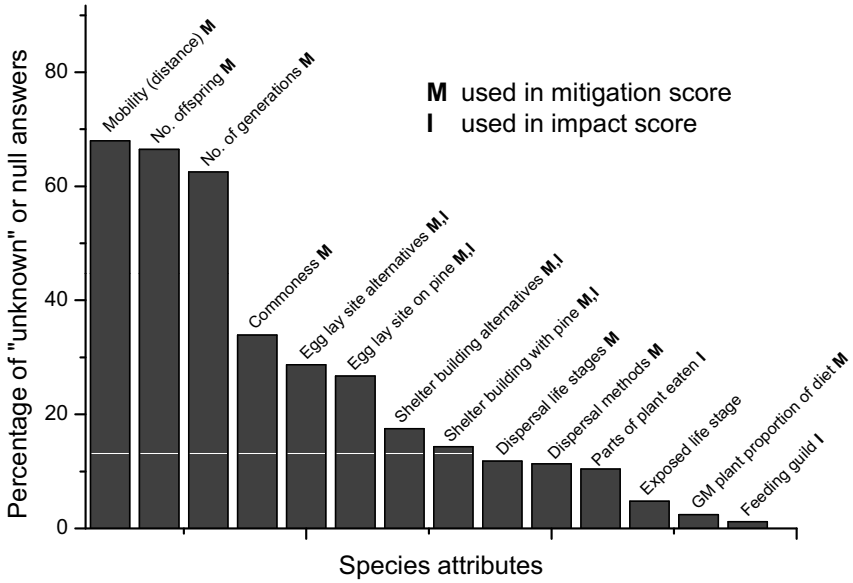

Figure 4. Uncertainty associated with our knowledge of attributes of invertebrate species found in New Zealand *Pinus radiata* plantation forests as indicated by the proportion of "unknowns" in a database of published biological and ecological information on 251 of those species. Attributes marked "I" were used to calculate a score representing the impact of altered-lignin GM pine on the species; those marked "M" were used to calculate a score representing the species' ability to mitigate those impacts.

protection of natural resources important to them, are assured by the Treaty of Waitangi 1840 and most modern New Zealand legislation relating to environmental protection makes reference to this treaty. In other countries, environmental protection goals for plantation forests may include special consideration of endangered or "red list" species.

The Eco Invertebase records a number of species attributes relevant to New Zealand's values-driven species protection goals: conservation status, if the species is native, endemic, adventive or introduced, if it has a Māori name, is an ecosystem service provider, an indicator species, or a food species for non-pest fish, native birds, reptiles or mammals. Scores were assigned to these and an anthropocentric value score, V, calculated. This produced a very different ranked list of species from those obtained when considering only the potential impacts of GM altered-lignin pine. Some native carabids and cicadas with Māori names scored highest; for example *Amphipsalta cingulata* F. (kihikihi wawā; Williams, 1971), which is an endemic cicada eaten by several native bird species and has been used as a biodiversity indicator (Benge et al., 2006), and *Megadromus antarcticus* (Broun) (kurikuri; Parkinson, 2007), which is an endemic, predatory carabid beetle known to be eaten by native birds. Native species of foliage-feeding Lepidoptera and Coleoptera, some of them prey for native birds, and some with Māori names, occupied middle positions on the list. Most lowly ranked were introduced or adventive species not known to provide food for other animals or to perform other ecosystem services, *e.g.,* the adventive wood wasp *Sirex noctilio* F., which is a pest with no Māori name and not recorded as being food for native animals (Miller, 1971).

When combined with the lignin impact score, F, as FV = F + V/10, the resultant newly ranked list of species (Tab. 3, "RankFV") showed significant movements in the places of some species compared with their ranking according to F but, in general, the list still bore a resemblance to the original (Tab. 3, "RankF"). Of the original "top 50" species, only four had changed. *Sirex noctilio*, *Apoctena orthropis* (Meyrick), *Navomorpha lineata* F. and *Ctenopseustis herana* Walker had been replaced by the endemic cicadas *A. cingulata* and *Amphipsalta zealandica* (Boisduval), the trichopteran *Triplectides cepaholotes* (Walker), and the lepidopteran *Liothula* Fereday sp.

Obviously, greater weight could be given to V if desired by reducing the numerator in the calculation of FV. For example, using FV = F + V/3 to rank species resulted in 38 species changes among the "top 50" compared with the original list ranked according to F. The reduction of V by a factor of 10 (or 3) was an arbitrary choice. V could theoretically be as high as 50, which would make it a very significant factor in the ranking of species compared with F. The inclusion of anthropocentric values in environmental risk assessments is unavoidable, because environmental management goals themselves are human-generated, but the extent to which these values drive the selection of test species for risk assessment of GM plants will vary from country to country depending on the relevant local legislation and public concerns. V's influence on test species selection can be

https://doi.org/10.1051/ebr/2011109 Published online by Cambridge University Press

adjusted accordingly by applying different weighing factors to suit local conditions.

Recommendations for measuring the impacts of altered-lignin GM pine on invertebrates

The original PRONTI model (Todd et al., 2008) was developed for a GM Bt plant application and has been adapted for other traits with obvious hazards or benefits for invertebrates (B.I.P. Barratt, pers. comm.). In contrast, the present model addressed lignin alteration, a trait that could confer an advantage, pose a hazard or have no effect, depending on the organism in question. For this reason, the concept of "impact" rather than "hazard" or "benefit" was used in this model. We did not include the PRONTI criteria of "ecological status", based on biomass and numbers of food web links, or "testability" of species, based on the availability of techniques for field-collecting, rearing and conducting bioassays, in this model. We were concerned that, with the current dataset of invertebrate species in pine forests, the PRONTI "ecological status" calculation heavily favoured well-studied species with many known food species and "downgraded" some that might well be affected by the modification but needed further study. As recommended by others (EFSA, 2010a; b; Romeis et al., 2011), we applied the pragmatic criterion of "testability" only after the model had generated the list of species ranked according to F and V. This took into account the practical consideration of efficient use of resources for experiments and gave us a means of distinguishing among taxonomically and functionally similar species.

We also applied a final pragmatic criterion of "taxonomic representation". Our current state of knowledge of the effects of lignin on insects is poor, but it is not unreasonable to assume that arthropod species belonging to different taxonomic groups might have fundamental differences in their physiology, morphology and symbiotic associations that affect their responses to lignin. Thus, if all other considerations are equal for a set of species, and we have resources to test only two, we suggest that more useful information on risk can be obtained if two species belonging to different taxonomic groups are tested, rather two belonging to a single taxonomic group. For example, tests with a weevil and a longhorn beetle species would be more informative than tests with two weevil species. As our knowledge of the effects of lignin on different taxa increases, the appropriateness of this taxonomically-based criterion could be reviewed.

To investigate hypothesised risks from planting altered-lignin GM pines (Tab. 2) using invertebrate species found in New Zealand pine plantations that are considered to be most likely to be affected, least likely to be able to avoid or mitigate the effect, and of most value, according to New Zealand environmental protection legislation (Tab. 3), we suggest the following experiments, in order of importance:

i) A comparison of the growth and survival of longhorn beetles, weevils and termites that feed on, tunnel in, and lay their eggs in decaying pine logs, on altered-lignin GM pine and isogenic non-GM control pine. The native cerambycid, *P. reticularis* (ranked 2 in Tab. 3), which is common, endemic and valued by Māori, and for which there is a recorded rearing technique (Rogers et al., 2002), would be a suitable candidate. Among the dead-wood-boring curculionids, perhaps the long-nosed kauri weevil, *Mitrastethus baridioides* Redtenbacher (ranked 6 in Tab. 3), which is a common native species, might be suitable, although a bioassay method would need to be developed for it. *Hylastes ater* Erichson (ranked 16 in the full version of Tab. 3) is another wood-boring weevil, which although not native, has the advantage of some published bioassay techniques (Zhang et al., 2004). *Euophryum rufum* (Broun), which tops the F and FV rankings in Tab. 3, is a rare species with no known rearing method and so would not be suitable. Of the termite species, perhaps *Stolotermes ruficeps* Brauer (ranked 13), a common endemic species which has been kept as an observation colony for three months (Morgan, 1959), would be a good choice. For these experiments, rotting wood of GM pine, and therefore probably large trees grown outdoors, would be required. These insects have long life cycles and conducting these experiments would not be a trivial task.

ii) A comparison of the growth and survival of a live-wood-chewing beetle on altered-lignin GM pine and isogenic non-GM control pine. The very common endemic cerambycid *Oemona hirta* (F.) (ranked 14) could be an amenable test subject for this. Bioassays have been conducted with this insect kept on twigs (Wang et al., 2002) and this method might be adapted for use with small glasshouse-grown pines.

iii) A comparison of the growth and survival of a leafroller (tortricid), which feeds on foliage and builds shelters using pine needles, on altered-lignin GM pine and isogenic non-GM control pine. The common native leafroller, *Planotortrix notophaea* (Turner) (ranked 36), would be a suitable candidate for this, as there is a well-established laboratory colony available at Plant & Food Research in New Zealand and a bioassay method using pine needles has been established (E.P.J. Burgess, unpublished).

https://doi.org/10.1051/ebr/2011109 Published online by Cambridge University Press

iv) A comparison of the growth and survival on altered-lignin GM pine and isogenic non-GM control pine of a species that feeds on dead pine needles or detritus. The endemic stick caddis *T. cephalotes* (ranked 44), which is common in still water and has a high reproductive output (Cowley, 1978), might be a suitable test organism, although a bioassay would have to be developed first.

Although we identified a potentially high risk of the GM pines having an impact on root-chewing insects (Tab. 2), among the top 50 species on our prioritised list of New Zealand pine-plantation-dwelling arthropods only *H. ater* has been recorded as feeding on roots of seedlings (and healthy plants can resist this damage) (Milligan, 1978). This suggests that this potential risk is less likely in this receiving environment than expected. The cicada (*Amphipsalta*) species on the list have underground nymphs that suck on xylem fluid and would not necessarily be as affected as a root-chewer by changes in lignin. The only root-chewing insects on the full list of 251 species known to occur on *P. radiata* pines were the scarab beetles *Costelytra zealandica* (White), *Odontria sylvatica* Broun, *Stethaspis lineata* (Arrow) and *Stethaspis suturalis* (F.), whose larvae can sometimes cause damage to pine seedling roots in nurseries and are controlled by insecticide applications. These scarabs were ranked between 92 and 132 on the full list of species, as they are not primarily wood-feeders, do not depend on only pine trees, and have been recorded from other habitats outside pine plantations.

Assessing impacts of altered-lignin GM pine on micro-organisms

The diversity of microbes in plant production systems and the poor state of our knowledge of their biology and ecology severely limits our ability to develop a test species selection procedure similar to that described above for invertebrates. However, some preliminary experiments with microbial species known to be important in New Zealand pine plantations, and for which there are some laboratory-handling techniques established, may be useful.

Many (but not all) forest fungi produce ligninolytic enzymes (laccases) that can break down different types of lignin and so this could lessen the potential impact of lignin alterations on some species of wood- or foliage-penetrating fungi (Lahtinen et al., 2009). For example, white-rot fungi are known to break down lignin; in fact, they are used commercially to de-lignify wood for pulping, but brown-rot fungi are known as poor degraders of lignin (Ferraz et al., 2001). Biodegradation tests with *P. radiata* wood and a series of New Zealand isolates of white-rot fungi would be useful for assessing the likelihood that lignin alteration could affect this important group of micro-organisms (see Ferraz et al. (2001) and Garrett et al. (2010) for potential testing techniques). Field-based studies to compare decay rates of wood and pine needles from each type of tree could also be carried out, even without knowledge of the actual organisms involved. It is possible that altered-lignin GM pines could exert selection pressure for strains of white-rot fungi best able to digest the new wood, and that the composition of fungal communities could change to minimise the overall impact on wood decomposition rates in the field. Researchers investigating impacts of GM plants on microbes are increasingly calling for the use of functional markers of important microbe-driven ecological processes rather than measures of species or genetic diversity in these communities (*e.g.*, Lottmann et al., 2010). The potential impacts of altered-lignin GM pines on decomposition and other microbial ecosystem functions will probably be best assessed by long-term field trials using measures that indicate significant changes, not in the populations of particular species, but in the functions they perform.

The durability of timber harvested from altered-lignin GM pines will undoubtedly be examined before commercialisation, to ensure that the desired new wood quality traits perform as expected. This should include assessment of "biological durability", *i.e.* the interactions between the timber and wood-rotting fungi in the built environment (buildings). The species and strains involved in wood-rotting at this stage may differ markedly from those in the forest, presenting a new aspect to ecological risk assessment for GM forest trees that does not have to be considered for other GM plants (R. Franich, pers. comm.).

The habits of different fungal species may also influence the likelihood that altering lignin will affect them. For example, the needle blight pathogens *Dothistroma septosporum* (Dorog.) Morelet (referred to in earlier reports as *D. pini*) and *Cyclaneusma minus* (Butin) Dicosmo, Peredo & Mint both enter pine needles *via* the stomata, but only *C. minus* can colonise the leaf tissue and even then, only to a limited extent (M. Dick, pers. comm.), suggesting that *D. septosporum* may be less affected by cell wall changes resulting from genetic modification to alter lignin properties. Schwelm et al. (2009) describe testing techniques that could be used with these fungi.

The root-rot pathogens *Armillaria novae-zelandiae* (G. Stev.) Boesew. and *Armillaria limonea* (G. Stev.) Boesew. are likely to produce ligninolytic enzymes (Stoytchev and Nerud, 2000) and so perhaps not be

https://doi.org/10.1051/ebr/2011109 Published online by Cambridge University Press

affected by lignin modifications, but this could be checked by exposing rooted cuttings of GM and non-GM pine to these fungi as described by Hood et al. (2009).

Neonectria fuckeliana (C. Booth) Castl. & Rossman has recently been found causing "nectria flute canker" in *P. radiata* in New Zealand (Crane et al., 2009). The biology of this species is not well known but the inclusion of a tree-inoculation test with this fungus when altered-lignin GM pines are field-tested would be advisable in order to determine whether susceptibility to this pathogen has been altered by the modification. Preliminary investigations to see if cultured *N. fuckeliana* possesses genes for or secretes laccases (Ferrer et al., 2010) could also provide useful information for risk assessment.

Various moulds, feeding on sugars and other compounds in the cambium, can cause sapstain in pine timber in New Zealand (Schirp et al., 2003). However, these do not penetrate the cell wall and so are unlikely to be affected by lignin modifications in GM pines (R. Franich, pers. comm.).

Pinus radiata's suite of ectomycorrhizal fungi in New Zealand is still being described, with 36 taxa identified so far (Chuchou and Grace, 1983; 1988; 1990; Walbert et al., 2010). As these fungi do not penetrate the tree's cell walls, lignin alterations are unlikely to affect them directly.

CONCLUSIONS

The approach to risk assessment used in this case study comprised a systematic consideration of organisms in the receiving environment and their prioritisation as test subjects according to a set of pre-determined criteria. These selection criteria were based on the likely impacts of the GM plant as a stressor (given the current state of knowledge), combined with the value of each receptor species' contribution to the achievement of environmental management goals. This approach might also be useful for other "metabolically modified" GM plants, which have no obvious "targets" and therefore no "non-targets" to consider, but could nevertheless have the potential to exert a significant influence on their receiving ecosystem. Our recommendations for experiments with altered-lignin GM pine took into account firstly, risk to and value of the test subjects, and then, if there was a choice of equivalent species, the ease with which practical tests could be conducted. The experiments recommended in this case study present the opportunity to build a useful body of knowledge for assessing impacts of other GM trees with lignin alterations and ensuring that field-based tests and post-release monitoring schemes for these plants have a sound theoretical basis.

METHODS

In this study the terms "assessment endpoints", "environmental management goals" and "conceptual models" are used as defined in the USEPA's "Guidelines for Ecological Risk Assessment" (USEPA, 1998).

The Eco Invertebase is a database of biological and ecological information on New Zealand invertebrate species developed using Microsoft® Access 2003 as described by Todd et al. (2008). For the present study, egg-laying and shelter-building behaviour and sites were added to the receptor attributes listed in Appendix 1 of Todd et al. (2008). Where there was no species-specific published information on these attributes, published descriptions of family characteristics were used as a guide.

Altered-lignin GM pine was added to the Eco Invertebase as a new stressor and the following species attributes recorded in relation to it: use of the stressor by the receptor (*e.g.*, as a direct food source (for herbivores and omnivores) or an indirect food source (for omnivores and predators or parasitoids)), proportion of the receptor's diet that is likely to be the stressor or a prey item exposed to and affected by the stressor, parts of the stressor eaten by the receptor, parts of the stressor affected by the genetic modification, likelihood that the receptor will ingest an affected part of the stressor or eat another organism that has been affected by it, receptor life stages potentially exposed to the stressor, and how the receptor may use the stressor as an egg-laying site or source of shelter-building materials and whether it has alternatives for these purposes.

Prioritised lists of species were derived from interrogation of the database using a series of queries written using Microsoft® Access 2003 or 2007 and further manipulated in spreadsheets using Microsoft® Excel 2007.

The species score is given by $F = I - (M/10)$, where I is an *impact score* and M a *mitigation factor*. A species score incorporating anthropocentric values is given by $FV = F + (V/10)$, where V is the *anthropocentric value*. *Impact score* (I) is the sum of Feed, Egglay, and Shelter, which are defined as follows:

Feed (for herbivores) is a score for lignin content of plant parts directly eaten by the species = 0 for pollen or nectar, 5 for unknown parts, 7 for detritus in water or soil, 8 for leaves, new growth, reproductive parts, phloem or leaf litter, 9 for xylem or stems, and 10 for roots or wood (living or dead). For carnivores, an indirect score based on pine-feeding species known to be prey for the predator or parasitoid in question is used instead, and for omnivores, the average of the direct and indirect scores is used.

https://doi.org/10.1051/ebr/2011109 Published online by Cambridge University Press

EggLay is the sum of LigninEggLay and AltEggLay, where

LigninEggLay is a score for how much lignin may affect egg-laying (based on parts of plant where eggs are laid) = 0 if on surface of foliage or wood or if scatters eggs or not known to use pine at all or "other", 5 if unknown, and 10 if inside foliage or wood (living or dead),

AltEggLay = score for availability of alternative places for this species to lay eggs = 1 if on surface or inside foliage or wood (living or dead), 5 if unknown or "other", 9 if scatters eggs, and 10 if not known to use pine at all.

Shelter is the sum of LigninShelt and AltShelt, where

LigninShelt = score for how much lignin may affect shelter-building (based on parts of plant used to build shelters) = 0 if does not use pine or "other", 5 if unknown, and 10 if uses foliage, other plant parts, dead wood or detritus,

AltShelt = score for availability of alternative places for this species to shelter = 1 for using foliage, other plant parts or dead wood, 5 for "other" or unknown, 9 for using detritus, and 10 for not specifically using this pine for shelter-building.

Mitigation factor (M) is the sum of logFS, PC, SRS, ERS, logEco, Max(ModeDisp) , Max(DispDist), Com, Reprod and YrGen, which are defined as follows:

logFS = natural log (count of known food species for this species + 1),

PC = plant choices score = 0 if only feeds on pine, 1 if primarily feeds on pine, 2 if pine is major part of diet, 3 if pine is minor part of diet or if diet is unknown, 4 if diet is unknown but unlikely to feed on pine, and 5 if does not feed on pine or any vegetation,

SRS = shelter resilience score = Shelter + Max(ModeDisp) + Max(DispDist) + count of mobile life stages,

ERS = egg resilience score = EggLay + Max(ModeDisp) + Max(DispDist),

logEco = natural log(count of non-pine ecosystems from which species has been collected + 1) (null entry = 0),

Max(ModeDisp) = maximum score for mobility based on possible modes of dispersal for any life stage of a species = 2 for crawl, 3 for jump, 5 for unknown, 6 for "other", 8 for hitch-hike, windblown or drift, 9 for swim, and 10 for fly (null entry = 10, which is the estimated median score based on 251 species records),

Max(DispDist) = maximum score for mobility based on recorded maximum distances travelled by a species = 1 if < 10 m, 2 if 10 to 100 m, 5 if unknown, 6 if 100 m to 1 km, 7 if 1 to 10 km, 9 if 10 to 100 km, and 10 if > 100 km (null entry = 5, which is the estimated median based on 251 records of pine-forest-dwelling species),

Com = commonness score = 1 if rare, 5 if status is unknown, and 10 if common (null entry = 10, which is an estimated median based on 251 species records),

Reprod = score for approximate reproductive rate = 1 if tens of offspring per year, 5 if unknown, 6 if hundreds and 10 if thousands (null entry = 5, which is the estimated median based on 251 species records),

YrGen = score for approximate number of generations per year = 1 if less than one generation per year, 4 if one generation per year, 5 if unknown, and 10 if more than one generation per year (null entry = 5, which is the estimated median based on 251 species records).

Anthropocentric value (V) is the sum of MN, ESP, Native, Threat and Max(NativeFood), which are defined as follows:

MN = 10 if the species has a traditional Māori name (*e.g.*, huhu for *P. reticularis*), 7 if it has a Māori name that is a transliteration of an English name (*e.g.*, pi for honey bee), 5 if there is no known Māori name, and 0 if the species is adventive and there is no record of a Māori name,

ESP = 10 if the species has a known function as an ecosystem service provider (*e.g.*, a biocontrol agent, natural enemy or an indicator species), 5 if it is not certain if it provides a function or not, and 0 if it is known not to, or is not likely to, provide a service,

Native = 10 if the species is endemic or native to New Zealand, 5 if its status is unknown, and 0 if it is adventive, either deliberately or self-introduced,

Threat = 10 if the species is rare, endangered or threatened according to the New Zealand Threat Classification System (Hitchmough, 2002), 5 = if its abundance is unknown, and 0 if common,

Max(NativeFood) = the maximum value of NativeFood, where NativeFood = 10 for species which are known to be preyed on by native mammals, birds, reptiles or amphibians, 8 for species preyed on by non-pest fish, 5 for species with unknown or "other" predators, 3 for species preyed on by non-native birds, 2 for those preyed on by other invertebrates, and 0 for those preyed on by pest fish or invertebrates, or non-native mammals, reptiles or amphibians.

Received April 1st, 2011; accepted September 2, 2011.

ACKNOWLEDGEMENTS

We thank Drs Margaret Dick, Ian Hood, Lisa Berndt and Robert Franich of Scion for their valuable advice on forest microbes, insects and lignin. Thanks are also due to

Aliesha Kean of Plant & Food Research, who entered many species records into the Eco Invertebase database, and to other colleagues who provided valuable reviews of and editorial input into the manuscript. This work was financially supported by the New Zealand Foundation for Research, Science and Technology, contract C06X801.

REFERENCES

Andow DA (2011) Assessing unintended effects of GM plants on biological species. *J. Verbr. Lebensm.* **6** (Suppl 1): S119–S124

Andow DA, Hilbeck A (2004) Science-based risk assessment for nontarget effects of transgenic crops. *Bioscience* **54:** 637–649

Andow DA, Hilbeck A, Nguyen Van Tuat (2008) Environmental Risk Assessment of Genetically Modified Organisms. Volume 4. Challenges and opportunities with Bt cotton in Vietnam. Oxford. CABI International

Anon (2005a) B/BE/07/V2Belgian Biosafety Server

Anon (2005b) Land use by farm type. Ministry of Agriculture and Forestry. http://www.maf.govt.nz/statistics/land-use/

Anon (2007) Notification report, B/BE/07/VE, 30/11/2007. European Commission Joint Research Centre, Institute for Health and Consumer Protection. http://gmoinfo.jrc.ec.europa.eu/gmp_report.aspx?CurNot=B/BE/07/V2

Anon (2008) Advice of the Belgian Biosafety Advisory Council on the notification of B/BE/07/V2 of the VIB, Flanders Institute of Biotechnology, for deliberate release in the environment of genetically modified poplars with an altered wood composition for research and development. WIV-ISP/BAC/2008_733. 25/04/2008. http://www.bio-council.be/bac_advices.html; http://www.bio-council.be/docs/BAC_2008_733.pdf

Axelsson EP, Hjaltan J, Leroy CJ, Julkunen-Tiitto R, Wennstrom A, Pilate G (2010) Can leaf litter from genetically modified trees affect aquatic ecosystems? *Ecosystems* **13**: 1049–1059

Barraclough EI, Burgess EPJ, Philip BA, Wohlers MW, Malone LA (2009) Tri-trophic impacts of Bt-expressing transgenic pine on the parasitoid *Meteorus pulchricornis* (Hymenoptera: Braconidae) *via* its host *Pseudocoremia suavis* (Lepidoptera: Geometridae). *Biol. Control* **49**: 192–199

Barratt BIP, Todd JH, Burgess EPJ, Malone LA Developing biosafety risk hypotheses for invertebrates exposed to GM plants using conceptual food webs: a case study with elevated triacylglyceride levels in ryegrass. *Environ. Biosafety Res.*, in press

Benge J, Moller H, Logan D (2006) Cicada species in kiwifruit orchards. ARGOS Research Note [online] www.argos.org.nz

Bishop-Hurley SL, Zabkiewicz RJ, Grace L, Gardner RC, Wagner A, Walter C (2001) Conifer genetic engineering: transgenic *Pinus radiata* (D. Don) and *Picea abies* (Karst) plants are resistant to the herbicide Buster. *Plant Cell Rep.* **20**: 235–243

Boerjan W, Ralph J, Baucher M (2003) Lignin biosynthesis. *Annu. Rev. Plant Biol.* **54**: 519–546

Bradley KL, Hancock JE, Giardina CP, Pregitzer KS (2007) Soil microbial community responses to altered lignin biosynthesis in *Populus tremuloides* vary among three distinct soils. *Plant Soil* **294**: 185–201

Chuchou M, Grace LJ (1983) Characterization and identification of mycorrhizas of radiata pine in New Zealand. *Australian Forest Research* **13**: 121–132

Chuchou M, Grace LJ (1988) Mycorrhizal fungi of radiata pine in different forests of the North and South Islands in New Zealand. *Soil Biol. Biochem.* **20**: 883–886

Chuchou M, Grace LJ (1990) Mycorrhizal fungi of radiata pine seedlings in nurseries and trees in forests. *Soil Biol. Biochem.* **22**: 959–966

Clissold FJ, Sanson GD, Read J, Simpson SJ (2009) Gross vs. net income: How plant toughness affects performance of an insect herbivore. *Ecology* **90**: 3393–3405

Colbourne R, Kleinpaste R (1983) A banding study of North Island brown kiwis in an exotic forest. *Notornis* **30**: 109–124

Cowley DR (1978) Studies on the larvae of New Zealand Trichoptera. *N. Z. J. Zool.* **5**: 639–750

Crane PE, Hopkins AJM, Dick MA, Bulman LS (2009) Behaviour of *Neonectria fuckeliana* causing a pine canker disease in New Zealand. *Can. J. For. Res.-Rev. Can. Rech. For.* **39**: 2119–2128

Dennis J, Kaplan I, Chu A (2005) Production and markets. In Colley M, ed, New Zealand Institute of Forestry Inc. Forestry Handbook. Tauranga, New Zealand, Design & Print Management Ltd, pp 190–192

ERMANZ, Environmental Risk Management Authority New Zealand (2010) Application for field testing genetically modified organisms in containment under section 40(1)(c) of the HSNO Act 1996. In: New Zealand Forest Research Institute Ltd taS (Ed.) Field Test in Containment Genetically Modified Pine Trees. Wellington, New Zealand, ERMANZ. pp. 57

EFSA, European Food Safety Authority (2010a) EFSA Panel on Genetically Modified Organisms (GMO); Guidance on the environmental risk assessment of genetically modified plants. *EFSA J.* **8**(11):1879. [111 pp.]. doi:10.2903/j.efsa.2010.1879

EFSA, European Food Safety Authority (2010b) EFSA Panel on Genetically Modified Organisms (GMO); Scientific opinion on the environmental risk assessment of genetically modified plants. *EFSA J.* **8**(11):1877 [72 pp.]. doi:10.2903/j.efsa.2010.1877

Ferraz A, Rodriguez J, Freer J, Baeza J (2001) Biodegradation of *Pinus radiata* softwood by white- and brown-rot fungi. *World J. Microbiol. Biotechnol.* **17**: 31–34

Ferreira SA, Pitz KY, Manshardt R, Zee F, Fitch M, Gonsalves D (2002) Virus coat protein transgenic papaya provides practical control of *Papaya ringspot virus* in Hawaii. *Plant Dis.* **86**: 101–105

Ferrer M, Beloqui A, Golyshin PN (2010) Screening metagenomic libraries for laccase activities. *Methods Mol. Biol.* **668**: 189–202

Find JI, Charity JA, Grace LJ, Kristensen MMMH, Krogstrup P, Walter C (2005) Stable genetic transformation of embryogenic cultures of *Abies nordmanniana* (Nordmann fir) and regeneration of transgenic plants. *In Vitro Cell. Dev. Biol.Plant* **41**: 725–730

Franich RA, Carson MJ, Carson SD (1986) Synthesis and accumulation of benzoic acid in *Pinus radiata* needles in response to tissue injury by dothistromin, and correlation with resistance of *Pinus radiata* families to *Dothistroma pini*. *Physiol. Mol. Plant Pathol.* **28**: 267–286

Garrett LG, Kimberley MO, Oliver GR, Pearce SH, Paul TSH (2010) Decomposition of woody debris in managed *Pinus radiata* plantations in New Zealand. *For. Ecol. Manag.* **260**: 1389–1398

Geib SM, Filley TR, Hatcher PG, Hoover K, Carlson JE, Jimenez-Gasco MD, Nakagawa-Izumi A, Sleighter RL, Tien M (2008) Lignin degradation in wood-feeding insects. *Proc. Natl. Acad. Sci. U. S. A.* **105**: 12932–12937

Grabber JH, Schatz PF, Kim H, Lu F, Ralph J (2010) Identifying new lignin bioengineering targets: 1. Monolignol-substitute impacts on lignin formation and cell wall fermentability. *BMC Plant Biology* **10**: 114

Grace LJ, Charity JA, Gresham B, Kay N, Walter C (2005) Insect-resistant transgenic *Pinus radiata*. *Plant Cell Rep.* **24**: 103–111

Hancock JE, Bradley KL, Giardina CP, Pregitzer KS (2008) The influence of soil type and altered lignin biosynthesis on the growth and above and belowground biomass allocation of *Populus tremuloides*. *Plant Soil* **308**: 239–253

Henault C, English LC, Halpin C, Andreux F, Hopkins DW (2006) Microbial community structure in soils with decomposing residues from plants with genetic modifications to lignin biosynthesis. *FEMS Microbiol. Lett.* **263**: 68–75

Hilbeck A, Andow DA (2004) Environmental Risk Assessment of Genetically Modified Organisms. Volume 1. A case study of Bt maize in Kenya. Oxford. CABI International

Hilbeck A, Andow DA, Fontes EMG (2006) Environmental Risk Assessment of Genetically Modified Organisms. Volume 2. Methodologies for assessing Bt cotton in Brazil. Oxford. CABI International

Hitchmough R (comp.) (2002) New Zealand Threat Classification System lists – 2002. *Threatened species occasional publication 23,* Department of Conservation, Wellington, New Zealand

Hood IA, Kimberley MO, Gardner JF (2009) Susceptibility to *Armillaria novae-zelandiae* among clones of *Pinus radiata*. *For. Pathol.* **39**: 405–414

Hotter GS (1997) Elicitor-induced oxidative burst and phenylpropanoid metabolism in *Pinus radiata* cell suspension cultures. *Aust. J. Plant Physiol.* **24**: 797–804

Huang DF, Zhang J, Song FP, Lang ZH (2007) Microbial control and biotechnology research on *Bacillus thuringiensis* in China. *J. Invertebr. Pathol.* **95**: 175–180

Huntley SK, Ellis D, Gilbert M, Chapple C, Mansfield SD (2003) Significant increases in pulping efficiency in C4H-F5H-transformed poplars: Improved chemical savings and reduced environmental toxins. *J. Agric. Food Chem.* **51**: 6178–6183

Johnson SN, Hallett PD, Gillespie TL, Halpin C (2010) Below-ground herbivory and root toughness: a potential model system using lignin-modified tobacco. *Physiol. Entomol.* **35**: 186–191

Kim KW, Lee IJ, Thoungchaleun V, Kim CS, Lee DK, Park EW (2009) Visualization of wound periderm and hyphal profiles in pine stems inoculated with the pitch canker fungus *Fusarium circinatum*. *Microsc. Res. Tech.* **72**: 965–973

Lahtinen M, Kruus K, Heinonen P, Sipila J (2009) On the reactions of two fungal laccases differing in their redox potential with lignin model compounds: products and their rate of formation. *J Agric Food Chem* **57**: 8357–8365

Levee V, Major I, Levasseur C, Tremblay L, MacKay J, Seguin A (2009) Expression profiling and functional analysis of *Populus* WRKY23 reveals a regulatory role in defense. *New Phytol.* **184**: 48–70

Lottmann J, O'Callaghan M, Baird D, Walter C (2010) Bacterial and fungal communities in the rhizosphere of field-grown genetically modified pine trees (*Pinus radiata* D.). *Environ. Biosafety Res.* **9**: 25–40

Miller D (1971) Common Insects in New Zealand. Wellington, A.H. & A.W. Reed Ltd

Milligan RH (1978) *Hylastes ater* (Paykull) (Coleoptera: Scolytidae). Black pine bark beetle. *New Zealand Forest Service, Forest and Timber Insects in New Zealand* **29**

Morgan FD (1959) The ecology and external morphology of *Stolotermes ruficeps* Brauer (Isoptera: Hodotermitidae). *Trans. Roy. Soc. N. Z.* **86**: 155–195

Nanayakkara B, Manley-Harris M, Suckling ID, Donaldson LA (2009) Quantitative chemical indicators to assess the gradation of compression wood. *Holzforschung* **63**: 431–439

Parfitt RL, Newman RH (2000) ^{13}C NMR study of pine needle decomposition. *Plant Soil* **219**: 273–278

Parkinson B (2007) A photographic guide to the insects of New Zealand. Auckland, New Holland Publishers (NZ) Ltd

Pawson SM, Ecroyd CE, Seaton R, Shaw WB, Brockerhoff EG (2010) New Zealand's exotic plantation forests as habitats for threatened indigenous species. *N. Z. J. Ecol.* **34:** 342–355

Peeters PJ, Sanson G, Read J (2007) Leaf biomechanical properties and the densities of herbivorous insect guilds. *Funct. Ecol.* **21**: 246–255

Pilate G, Guiney E, Holt K, Petit-Conil M, Lapierre C, Leple JC, Pollet B, Mila I, Webster EA, Marstorp HG, Hopkins DW, Jouanin L, Boerjan W, Schuch W, Cornu D, Halpin C (2002) Field and pulping performances of transgenic trees with altered lignification. *Nat. Biotechnol.* **20**: 607–612

https://doi.org/10.1051/ebr/2011109 Published online by Cambridge University Press

Prins RA, Kreulen DA (1991) Comparative aspects of plant-cell wall digestion in insects. *Anim. Feed Sci. Tech.* **32**: 101–118

Punelli F, Reverberi M, Porretta D, Nogarotto S, Fabbri AA, Fanelli C, Urbanelli S (2009) Molecular characterization and enzymatic activity of laccases in two *Pleurotus* spp. with different pathogenic behaviour. *Mycol. Res.* **113**: 381–387

Raybould A, Caron-Lormier G, Bohan D (2011) Derivation and interpretation of hazrd quotients to assess ecological risks from the cultivation of insect-resistant transgenic crops. *J. Agric. Food Chem.* **59:** 5877–5885

Rogers DJ, Lewthwaite SE, Dentener PR (2002) Rearing huhu beetle larvae, *Prionoplus reticularis* (Coleoptera: Cerambycidae) on artificial diet. *N. Z. J. Zool.* **29**: 303–310

Romeis J, Hellmich RL, Candolfi MP, Carstens K, De Schrijver A, Gatehouse AMR, Herman RA, Huesing JE, McLean MA, Raybould A, Shelton AM, Waggoner A (2011) Recommendations for the design of laboratory studies on non-target arthropods for risk assessment of genetically engineered plants. *Transgenic Res.* **20**: 1–22

Schirp A, Farrell RL, Kreber B (2003) Effects of New Zealand sapstaining fungi on structural integrity of unseasoned radiata pine. *Holz Als Roh-und Werkst.* **61**: 369–376

Schnitzler F-R, Burgess EPJ, Kean AM, Philip BA, Barraclough EI, Malone LA, Walter C (2010) No unintended impacts of transgenic pine (*Pinus radiata*) trees on above-ground invertebrate communities. *Environ. Entomol.* **39**: 1359–1368

Schwelm A, Barron NJ, Baker J, Dick M, Long PG, Zhang S, Bradshaw RE (2009) Dothistromin toxin is not required for dothistroma needle blight in *Pinus radiata*. *Plant Pathol.* **58**: 293–304

Seppanen SK, Pasonen HL, Vauramo S, Vahala J, Toikka M, Kilpelainen I, Setala H, Teeri TH, Timonen S, Pappinen A (2007) Decomposition of the leaf litter and mycorrhiza forming ability of silver birch with a genetically modified lignin biosynthesis pathway. *Appl. Soil Ecol.* **36**: 100–106

Stoytchev I, Nerud F (2000) Ligninolytic enzyme complex of *Armillaria* spp. *Folia Microbiol.* **45**: 248–250

Sutela S, Niemi K, Edesi J, Laakso T, Saranpaa P, Vuosku J, Makela R, Tiimonen H, Chiang VL, Koskimaki J, Suorsa M, Julkunen-Tiitto R, Haggman H (2009) Phenolic compounds in ectomycorrhizal interaction of lignin modified silver birch. *BMC Plant Biol.* **9**: 124

Tiimonen H, Aronen T, Laakso T, Saranpaa P, Chiang V, Haggman H, Niemi K (2008) *Paxillus involutus* forms an ectomycorrhizal symbiosis and enhances survival of PtCOMT-modified *Betula pendula in vitro*. *Silvae Genet.* **57**: 235–242

Tiimonen H, Aronen T, Laakso T, Saranpaa P, Chiang V, Ylioja T, Roininen H, Haggman H (2005) Does lignin modification affect feeding preference or growth performance of insect herbivores in transgenic silver birch (*Betula pendula* Roth)? *Planta* **222**: 699–708

Todd JH, Ramankutty P, Barraclough EI, Malone LA (2008) A screening method for prioritizing non-target invertebrates for improved biosafety testing of transgenic crops. *Environ. Biosafety Res.* **7**: 35–56

Tronchet M, Balague C, Kroj T, Jouanin L, Roby D (2010) Cinnamyl alcohol dehydrogenases-C and D, key enzymes in lignin biosynthesis, play an essential role in disease resistance in *Arabidopsis*. *Mol. Plant Pathol.* **11**: 83–92

Uprichard JM (1991) Chemistry of Wood and Bark. In Kininmonth JA, Whitehouse LJ, eds, Properties and Use of Radiata Pine, Vol 1 - Wood Properties. Rotorua, New Zealand, New Zealand Ministry of Forestry, Forest Research Institute

USDA (2010) Permit applications 08–011–106rm and 08–014–101rm received from ArborGen LLC. Field testing of genetically engineered *Eucalyptus grandis* X *Eucalyptus urophylla*. Final Environmental Assessment. April 2010

USEPA, United States Environmental Protection Agency (1998) Guidelines for Ecological Risk Assessment. EPA/630/R095/002F. http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=12460

Valenzuela S, Balocchi C, Rodriguez J (2006) Transgenic trees and forestry biosafety. *Electron. J. Biotechnol.* **9**: 335–339

Vanholme R, Morreel K, Ralph J, Boerjan W (2008) Lignin engineering. *Curr. Opin. Plant Biol.* **11**: 278–285

Wagner A, Donaldson L, Kim H, Phillips L, Flint H, Steward D, Torr K, Koch G, Schmitt U, Ralph J (2009) Suppression of 4-coumarate-CoA ligase in the coniferous gymnosperm *Pinus radiata*. *Plant Physiol.* **149**: 370–383

Wagner A, Ralph J, Akiyama T, Flint H, Phillips L, Torr K, Nanayakkara B, Kiri LT (2007) Exploring lignification in conifers by silencing hydroxycinnamoyl-CoA : shikimate hydroxycinnamoyltransferase in *Pinus radiata*. *Proc. Natl. Acad. Sci. U. S. A.* **104**: 11856–11861

Walbert K, Ramsfield TD, Ridgway HJ, Jones EE (2010) Ectomycorrhizal species associated with *Pinus radiata* in New Zealand including novel associations determined by molecular analysis. *Mycorrhiza* **20**: 209–215

Walter C, Fladung M, Boerjan W (2010) The 20-year environmental safety record of GE trees. *Nat. Biotechnol.* **28**: 656–658

Wang Q, Shi GL, Song D, Rogers DJ, Davis LK, Chen XN (2002) Development, survival, body weight, longevity, and reproductive potential of *Oemona hirta* (Coleoptera : Cerambycidae) under different rearing conditions. *J. Econ. Entomol.* **95**: 563–569

Watt JC (1982) New Zealand beetles. *N. Z. Entomol.* **7**: 213–221

Wilkinson M, Tepfer M (2009) Fitness and beyond: Preparing for the arrival of GM crops with ecologically important novel characters. *Environ. Biosafety Res.* **8**: 1–14

Williams HW (1971) Dictionary of the Maori Language, Seventh Edition. Wellington, Legislation Direct

Zhang Z, Van Epenhuijsen CW, Brash D, Hosking GP (2004) Phosphine as a fumigant to control *Hylastes ater* and *Arhopalus ferus*, pests of export logs. *N. Z. Plant Protection* **57**: 257–259

https://doi.org/10.1051/ebr/2011109 Published online by Cambridge University Press