Genetic Engineering of Trees to Enhance Resistance to Insects

Evaluating the risks of biotype evolution and secondary pest outbreak

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Control of insect pests has often been suggested as a promising use of the emerging gene-insertion technology. The most commonly proposed strategies would improve microbial pathogens of insects, incorporate defects into pest populations, and transfer genes that encode for insect-resistance properties into plants. Although some advantages and risks of these strategies have been discussed in general terms, there is little conceptual framework for applying theoretical principles to specific policy decisions (Simonsen and Levin 1988). In this article, I develop an approach to estimating risk by concentrating on one strategy—genetic transfer of resistance properties into otherwise susceptible hosts (Vaeck et al. 1987)—in one commodity frequently cited as a beneficiary of biotechnology—wood products (Dandekar et al. 1987, Farnum et al. 1983, Sederoff and Ledig 1985). Such an approach should improve our ability to evaluate and reduce the potential for inadvertent deleterious alteration of forest ecosystems. It may serve as a model for other transgenic applications.

I focus on one potential danger, biotype evolution, which is the selection for insect populations that can tolerate the new resistance property (Gould 1988). I do not consider here the impact of reduced insect populations on wildlife and nutrient cycling, problematic escaped plant material, and the great value of these technologies as research tools.

Rationale for genetic enhancement of tree resistance

Much of the impetus for transferring foreign genes into trees arises from the unsuitability of traditional pest control tactics. For example, use of insecticides, a staple of insect control in many annual crops, is limited in forestry, where it is often infeasible, ineffective, or environmentally unacceptable. Application costs can be high because forests occur in immense, often inaccessible tracts; trees require a large amount of vertical coverage; and protection must extend over many growing seasons, rather than just one. Moreover, forests are often intended for multiple uses such as recreation, watershed management, and grazing, in addition to wood production.

Most forest insect management programs are based on a combination of silvicultural (stand density, rotation schedule, species-age composition, and site selection) and biological controls. Although often effective, these tactics also have limitations. In many cases, environmentally sound and entomologically efficacious recommendations cannot be adopted because they are not cost efficient.

Breeding for resistance against insects has not been pursued in forestry to the same extent as in agriculture. Although heritable resistance to some pests has been identified, operational problems have precluded implementation. For example, difficulties associated with breeding large plants, which require long periods before sexual maturity, can be prohibitive and pose formidable barriers to a scientist’s career. Adding to this problem is the legacy of nineteenth-century high-grading, the cutting of only superior trees, which left defective and pest-infected specimens as the genetic base of present forests (Barrett 1980).

Genetic improvement of trees, whether by traditional methods or biotechnology, can be highly compatible with silvicultural and biological insect control tactics. The attractiveness of using gene alteration to accelerate tree improvement has also been heightened by the conversion to more intensive tree management. When most forests were relatively unmanaged, insect feeding did not necessarily translate into commercial losses because companies simply purchased tracts as they matured rather than actively cultivating trees. However, intensively managed plantations, seed orchards, and energy farms comprise more sizable grower investments. These growing conditions also favor the survival and reproduction of some insects, just as in agriculture (Scho-
walter 1985). The Technical Association for Pulp and Paper Industries predicted that genetically engineered pest resistance would provide the greatest improvement to be gained by biotechnology over current practices, with significant impact within 3–5 years (Stomp 1987).

Genetic engineering, however, could also impose novel and unprecedented selective pressures on forest insect populations. The anticipated efficiency and efficacy, as opposed to the practical limitations of insecticides and traditional resistance breeding, pose major risks that have not yet been addressed in forest ecosystems. Resistant insect biotypes cause, at the least, a loss of efficacy and product failure. However, some resistant biotypes could cause greater problems than existed before the novel genes were deployed. Because trees serve as both commercial and natural resources, and because naturally regenerating forests located near planted stands comprise major terrestrial ecosystems, these risks must be addressed before large-scale outplantings can be deemed judicious.

**General principles of biotype evolution**

Generally accepted principles have emerged from several well-established disciplines, particularly the study of pesticide resistance, crop breeding, and plant-insect coevolution, that can be applied to tree gene manipulation. First, there is no physiological mode of insecticide action, if applied with sufficient intensity, that cannot be overcome by insect populations. Synthetic insecticides include a wide variety of exotic molecules that the target organisms did not previously encounter in their evolutionary histories. Yet resistant races have emerged against all of them, often in manners that confer cross-resistance to related, and even unrelated, chemicals (Croft et al. 1982). So one cannot argue that genetically engineered resistance is immune to counteradapted biotypes because genes can be introduced from organisms unrelated to the host plant.

The pattern and intensity of selective pressures, rather than the actual mode of toxicity, most strongly affects the emergence of resistant races (Brattsten et al. 1987, Tabashnik and Cross-resistance can take unpredictable forms, including immunity against currently effective methods. For example, Gould et al. (1982) demonstrated that mine adaptations to resistant plant cultivars can decrease their susceptibility to organophosphates.

Previously innocuous nontarget insects can also be elevated to pest status through natural-enemy elimination, competitive release, and sometimes direct physiological benefit. Mite populations commonly rise after pyrethroid or DDT applications to which the target pest and mite natural enemies, but not the phytophagous mites, are susceptible. For example, the only major outbreak of the spruce spider mite, Oligonychus ununguis Jacobi, in natural forests followed aerial DDT sprays against the western spruce budworm, Choristoneura occidentalis Freeman (Furniss and Carolin 1977). Likewise, new plant cultivars have inadvertently created new pests by altering existing host-insect relationships (Oka and Bahagiawati 1984, Pathak 1975).

In some cases, the desired plant property, such as high yield or resistance to another pest, bestowed benefit to another herbivore in the form of direct nutritive properties or toxicity to parasites (Campbell and Duffy 1979). For example, the green revolution introduced several high-yielding cultivars that resulted in new pest complexes (Harlan 1980, Pathak 1975). Naturally coevolved systems also provide numerous examples where host defensive chemicals favor adapted herbivores by repelling predators (Eisner et al. 1974), inhibiting gut pathogens (Andrews et al. 1980), or providing a food base (Bernays and Woodhead 1982). Therefore, identical standards must be applied to trees genetically engineered for properties other than pest resistance, as any alteration of the host is likely to affect the selective pressures on closely adapted herbivores.

Third, biotype formation is not an occasional aberration, but rather an inevitable outcome of certain conditions. More than 428 arthropod species developed insecticide resistance by 1980 (Geourghiou and Mellon 1983), and 24 of the 25 major agricultural pests in California comprise either secondary pest outbreaks or

Red pine defoliated by Neodiprion sertifer. Photo by David Hall.

Croft 1982). Factors such as frequency of application, refugia among wild hosts, and persistence of additional selective pressures such as natural enemies are critical. Coevolutionary theory reinforces this lesson. In natural ecosystems, long-lived trees maintain defensive capacity against insects, despite the enormous differences in their generation times, partly because of the conflicting and varying selective pressures imposed by the aggregate environment (Edmunds and Alstad 1978, Fritz et al. 1986, Raffa and Berryman 1987, Whitham 1983).

Second, there are numerous examples in which new or more severe pest problems than occurred before treatment have arisen (Forgash 1984). A common sequence is reduction of the target insect’s natural enemies, followed by target insect resistance, followed by unchecked damage. Natural enemies usually evolve resistance more slowly than the target (Croft and Strickler 1983), and so new pesticides must be employed. The result is a classic pesticide treadmill. Increased target-pest problems can also follow the introduction of resistant plant varieties. For example, adaptations to resistant cultivars of a preferred crop species can enhance a pest’s ability to attack normally less-susceptible crops (Gould 1979).
Table 1. Mechanisms by which insect evolutionary responses to genetically altered trees could deplete efficacy, contribute to more severe problems with target pests, and create new pest problems. Each mechanism has been observed in response to insecticides and/or new plant cultivars. +: Outcome that could result from a particular mechanism. See text for explanation and examples.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Loss of efficacy</th>
<th>Aggravated target pest problems</th>
<th>Nontarget pest emergence</th>
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<tbody>
<tr>
<td>Physiological adaptation</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No cross-resistance with current tactics</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Cross-resistance with current tactics</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Cross-resistance between introduced and existing defenses in:</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Primary host</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Secondary hosts</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Natural enemy suppression by toxin</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Altered host availability patterns after biotype evolution:</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Primary pest threshold surpassed</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Predisposition to secondary pests</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Competitor elimination</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Selection for host-preference shifts</td>
<td>-</td>
<td>+</td>
<td>+</td>
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</table>

Pest resurgence facilitated by insecticides (Forgash 1984, Metcalf 1980). Immune biotypes have also emerged against such control practices as *Bacillus thuringiensis* (Mgaughhey 1985) application and alternate crop rotation (Krysan et al. 1986).

The potential mechanisms of insect response to and adverse consequences of genetic engineering in trees, and the mechanisms by which each could arise, are summarized in Table 1. Tree longevity intensifies these risks, because a single host generation may span several hundred insect generations, a full order of magnitude more than is normally required for insect biotypes to emerge against insecticides (Forgash 1984, Metcalf 1980) and resistant cultivars (Sosa 1981).

Given these possibilities, and the complexities of biological systems outside controlled laboratory conditions, one alternative is to conclude that the effects of tree gene manipulations simply cannot be predicted, and therefore implementation should be avoided. However, this carte blanche disapproval is itself fraught with risk: development and application will continue with or without the input of ecologists, and general reservations will simply not carry much weight as legal decisions are rendered, financial markets are explored, and ordinances from international to local levels are modified to compete for sources of jobs and revenue. Also, molecular biologists have been subjected to such a cacophony of hypothetical worst-case scenarios that specific concerns must be detailed, lest real dangers be likewise summarily dismissed. By suggesting ways of using ecological factors to lessen the risks of biotype evolution, forest ecologists can provide some direction to future decisions and research needs. Without such input, single-strategy approaches become more likely, and these approaches are surely the most detrimental.

The approach suggested here consists of developing the ability to characterize specific host-pest targets according to general levels of risk, devising tactics for reducing the chances of target and nontarget biotype evolution in systems deemed to have an acceptable level of risk, and initiating long-term strategies for fostering the environmentally safe use of plant genetic engineering in forestry.

**Selection of target systems**

Experiences with insecticides and resistant cultivars indicate that detailed knowledge of each target host/pest system is required before judicious decisions can be made. However, the enormous diversity of insect biologies, host physiologies, and tree-growing conditions makes a case-by-case appraisal unwieldy and undirected. Therefore, a framework for transferring general principles to specific evaluations of risk is presented. Four factors are considered: the availability of local refugia for susceptible insect genotypes, the existing role of host defenses in the pest's population dynamics, the compatibility of novel genetic defenses with alternative management practices, and the ability of novel genes to be transferred to plant progeny.

Refugia consist of untreated plants, plant parts, or times in which the herbivore can successfully feed and develop to maturity without exposure to the novel trait. Because area-wide, consistent selection pressures accelerate biotype formation, whereas spatially disrupted, intermittent exposure favors preservation or restoration of previous gene frequencies, an abundance of local refugia is critical. Application of this concept under actual field conditions is complicated, however, because refugia for susceptible genes are determined by the target insect's biology, host physiology, plant community structure, application patterns, and various interactions thereof (Table 2).

Mere proximity between different host types is not sufficient to preclude race formation (Bush 1973). Precautions must be taken to avoid reproductive isolation, and susceptible genes can only be preserved in systems where there is a high likelihood that some insects will locate untreated suitable hosts within their lifetime and interbreed with exposed individuals.

Host plant physiology, distribution, and variability strongly affect the population dynamics, behavior, and gene frequencies of herbivorous insects (Alstad and Edmunds 1983, Berryman 1976, Raffa and Berryman 1983, 1987). The consequences of genetic engineering on these naturally occurring constraints must be considered with respect to survival and reproductive rates by new insect races or nontarget species released from competitors and natural enemies. For example, if a counteradaptation also conferred cross-resistance to existing plant defenses, the consequences would be most severe in systems.
Table 2. Specific properties of target insect-tree systems that may influence the likelihood and severity of heritable insect responses to genetically engineered trees. For each property, conditions deemed more likely to yield reduced levels of risk are proposed.

<table>
<thead>
<tr>
<th>System properties</th>
<th>Attributes reducing risk</th>
</tr>
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<tbody>
<tr>
<td>Refugia: Opportunity for nonadapted insect survival</td>
<td></td>
</tr>
<tr>
<td>Plant community structure</td>
<td>High</td>
</tr>
<tr>
<td>Number of untreated trees</td>
<td></td>
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<tr>
<td>Insect life history</td>
<td></td>
</tr>
<tr>
<td>Mobility</td>
<td>High</td>
</tr>
<tr>
<td>Mating site</td>
<td></td>
</tr>
<tr>
<td>Number of trees oviposited by female</td>
<td>Distant from host</td>
</tr>
<tr>
<td>Fecondity</td>
<td>Many</td>
</tr>
<tr>
<td>Plant physiology</td>
<td>Low</td>
</tr>
<tr>
<td>Distribution and expression of trait</td>
<td>Varied</td>
</tr>
<tr>
<td>Insect life history *Plant community structure</td>
<td></td>
</tr>
<tr>
<td>Available acceptable species</td>
<td>High</td>
</tr>
<tr>
<td>Insect life history *Plant physiology</td>
<td>Low</td>
</tr>
<tr>
<td>Number of insect generations/tree growing cycle</td>
<td></td>
</tr>
<tr>
<td>Insect population regulation</td>
<td>Low</td>
</tr>
<tr>
<td>Effect of existing host defenses on insect reproduction</td>
<td>Nonlethal?</td>
</tr>
<tr>
<td>Effect of novel host plant defense on insect</td>
<td>Dissimilar</td>
</tr>
<tr>
<td>Action of existing and novel defense mechanisms</td>
<td></td>
</tr>
<tr>
<td>Compatibility of novel trait with other pest</td>
<td></td>
</tr>
<tr>
<td>management methods</td>
<td></td>
</tr>
<tr>
<td>Biological control</td>
<td></td>
</tr>
<tr>
<td>Host range of major natural enemies</td>
<td>Broad</td>
</tr>
<tr>
<td>Exposure of natural enemies to novel trait</td>
<td>Low</td>
</tr>
<tr>
<td>Tolerance of natural enemies to novel trait</td>
<td>High</td>
</tr>
<tr>
<td>Dispersal capability of natural enemies</td>
<td>High</td>
</tr>
<tr>
<td>Silvicultural control</td>
<td></td>
</tr>
<tr>
<td>Optimal insect strategy for coping with novel plant</td>
<td>Conflicting</td>
</tr>
<tr>
<td>trait and patterns of suitable host availability</td>
<td>High</td>
</tr>
<tr>
<td>Feasibility of insect removal by sanitation</td>
<td></td>
</tr>
<tr>
<td>Insecticidal control (synthetic and microbial)</td>
<td>High</td>
</tr>
<tr>
<td>Availability of cost-effective sprays</td>
<td></td>
</tr>
<tr>
<td>Mode of action of directly applied and plant-</td>
<td>Dissimilar</td>
</tr>
<tr>
<td>incorporated toxins</td>
<td></td>
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</table>

*Interaction between plant and insect properties.

Figure 1. By applying the specific traits for each system as outlined in Table 1, potential targets can be situated within general zones of risk. Potential targets will be addressed in a descending hierarchy, in which the cropping system is considered first. Within each cropping system, some major pests will be evaluated with emphasis on the parameters shown in Figure 1. The population behavior and management considerations pertaining to each pest are discussed in turn. The objective is not to address every important pest, but rather to provide examples of how specific cases can be evaluated.

Initially, biotype formation is considered for the target pest alone. This constraint is subsequently relaxed in the discussion on nontarget pest emergence. In practice, this distinction is arbitrary, because the tactics in the latter discussion must also be applied to each target pest.

Growing conditions

Commercial forests. Large commercial forests, consisting of either naturally regenerating stands or plantations, comprise a major portion of wood and fiber production. Plantations are usually even-aged monocultures derived from seed orchards, whereas the more expansive self-regenerating tracts vary in species, age, and genetic diversity, depending on their ecological status and logging practices. Ownership is distributed among government agencies, large corporations, and regional timber companies. Even though the total value of this resource is enormous, per-acre profits are low, and so traditional methods of insect control are...
not cost effective in these systems. Likewise, insecticides pose greater environmental hazards in large forests than in other tree-growing systems, because uses such as watershed management and recreation may exceed the importance of wood products. Although these factors lend support to genetic engineering in large forests, widespread, uninterrupted deployment of novel genes would post a high likelihood of biotype evolution. The availability of untreated refugia could be extremely small, because there would be little gene flow between exposed and unexposed insects, and harvest intervals are lengthier than for all other conditions except landscape ornament. Therefore, special precautions to preserve susceptible insect genes must be employed in these high-acreage commercial systems.

Short-rotation intensive cultivation. Recent advances in the development of fast-growing species, such as Populus and Salix, may lead to revolutionary changes in the tree-growing industries. In addition to providing traditional wood products, agriforestry promises to be a major source of energy. Selected cultivars have shown tremendous capacity for biomass production (Ek et al. 1983), and rotation cycles of only 1–10 years have been proposed. Currently, these trees are typically planted on low acreages under intensive management, and they may yield high cash values.

Short rotation times and intensive cultivation methods are well suited for the deployment of spatial and temporal genetic mosaics. By alternating resistant foreign genotypes, mixing clonal varieties, and integrating plant resistance with biological, cultural, and chemical controls in areas where untreated hosts are abundant, the chances of biotype formation can be reduced. If managed wisely, genetic engineering may even help preserve existing genes that confer protection from insects. The current intense selection and germplasm manipulation of fast-growing hardwoods poses the challenge of achieving superior production properties without sacrificing pest resistance (Harrell et al. 1981), as happened in food plants. Gene transfer methods may prove most efficient at combining such multiple traits.

Seed orchards and high-value plantations. Seed orchards are generally low-acreage, high-value, intensively managed sites. They are often surrounded by large forests which could provide a substantial reservoir of susceptible, intermingling insect genotypes. Because even low levels of insect feeding cause severe economic losses, biological controls are not always satisfactory. Likewise, cultural remedies may conflict with growing practices, just as they often do in agriculture. Because seed orchards must produce a consistently high yield, they provide a plentiful, predictable resource to specialized herbivores that have evolved high reproductive capacity as an adaptation to a naturally scarce, unpredictable food supply. High-value plantation trees (e.g., Christmas trees and veneer) pose approximately the same level of risk as seed orchards.

Ornamental trees. Trees grown for ornament and shade are among the most valuable on a per-individual basis, with large lawn trees contributing greatly to real estate values (Payne et al. 1973). Extensive monocultures are not common: usually an age and species mosaic is strewn across the suburban and urban landscape, throughout which untended woodlots are scattered and around which larger forests often occur.

Several features of this system could provide for the critically needed susceptible-insect gene refugia. First, the outlying regions from which urban pests often arise could be left unaltered, with alternative methods being used in the forests. Second, urban parks and woodlots could be left untreated and municipal foresters could continue current practices. Third, not all homeowners would choose any one variety of any one species. Introduction of foreign genes is already well underway in this setting, as some homeowners plant Japanese white birch to avoid bronze birch borer and Chinese elm to avoid Dutch elm disease. No biotype evolution has been reported. Genetic engineering in this context would simply be a more efficient and directed version of the same process.

Insect groups

Bark beetles. Bark beetles (Coleoptera: Scolytidae) are the most
damaging forest pests to mature trees (Coulson and Witter 1984). Silvicultural tactics can reduce losses, but no method is totally satisfactory. Therefore, these insects might seem a logical group to suppress by gene transfer.

Bark beetles, however, might cause even greater problems after biotype evolution than they do currently, because of their particular relationship with host plants. Individual trees within a host species vary greatly in their levels of resistance. All trees can resist low numbers of beetles by secreting repellent and toxic resins, but high attack densities mediated by aggregation pheromones can exhaust these defenses (Raffa and Berryman 1983, Rudinsky 1962). Thus the interaction is quantitative in that the outcome is dictated by the interaction between tree defense capabilities and insect numbers, but it is also discrete in that colonization attempts result in either tree death or insect failure to reproduce. Moreover, a tree’s threshold of resistance—the number of beetles required to overcome its defenses—is greatly diminished by environmental stresses, such as water deficit, disease, lightning, and other insects.

Under most conditions, even the most damaging bark beetle species usually attack stressed trees (Rudinsky 1962), because the ability to detect weakened hosts increases their chances of reproduction. If there is a sudden increase in susceptible trees due to an area-wide stress such as drought, however, the population can surpass a critical density above which beetle behavior changes (Berryman 1976, Raffa and Berryman 1987). Beetle densities can become high enough to overcome the resistance of almost all trees in the stand, even after the stress has been removed. Under these conditions, beetles functionally expand their own food supply by successfully attacking healthy trees, and devastating outbreaks occur. Thus bark beetle population levels relate closely to the vigor of their host plants (Berryman 1976). Natural enemies and temperature play important roles, but most forest managers associate beetle outbreaks with old trees, dense stands, and physical stress.

Effective bark beetle management maintains conditions that confine populations to a scavenging existence by promoting stand vigor through early rotation, thinning, sanitation, proper site selection, and age mosaics. Because beetles compete with other beetles (not trees), we need to provide conditions under which the best beetle strategy is to orient away from healthy trees, and individuals that are not repelled by healthy trees are at a competitive disadvantage (Raffa 1988).

This stability could be reduced by deployment of a foreign gene conferring resistance. If all trees, stressed and healthy alike, expressed the resistance factor, the selective pressures on beetle populations would be enormous and unidirectional. Behavioral attributes that orient beetles to weakened trees would confer little advantage. The best chance of beetle reproductive success would be offered by combined physiological immunity to both the novel and current tree defensive traits. With the role of host plant resistance removed, no effective control strategies would remain. Although dissimilarities between natural and exotic plant defense mechanisms can reduce this possibility, they do not provide sufficient assurance.

Even if beetles only overcame the novel gene but not natural resistance mechanisms, damaging conditions could still result. An accumulated pool of previously protected, stressed trees would become available, allowing populations to rise above outbreak thresholds. A widespread, synchronous appearance of suitable hosts, as opposed to a chronic, scattered reservoir of stricken trees, greatly increases the likelihood of damaging bark beetle outbreaks (Raffa and Berryman 1987). The pattern would be similar to what has already proven to be a failed, now abandoned, fire control strategy: by suppressing small fires, managers allowed tinder to accumulate to unnatural volumes, and uncontrollable, devastating fires resulted.

Transgenic trees might also suppress natural enemy populations, because most arthropod predators and parasites of scolytids complete development within trees killed by the beetles. Once beetles evolved resistance to the novel property, not only would there be an enormous backlog of available hosts, but there could also be a scarcity of natural enemies. Although not the principal factors regulating scolytid abundance, these arthropods are important mortality agents, in whose absence tree losses would increase (Amman and Cole 1983). Moreover, adverse effects would probably spread beyond treated stands, as emigrants from outbreaks often damage neighboring forests. There are no effective measures against such high populations (Berryman 1976).

Mosaic strategies cannot provide sufficient assurance that this threat would be alleviated. Leaving some trees untreated would mostly provide a reservoir of vigorous plants, and so would not maintain current selective advantages to beetles that orient to unhealthy trees. Biotype formation could theoretically be delayed by deliberately stressing untreated trees, but this approach demands a level of precision that far exceeds both current scientific capabilities and anticipated operational fidelity. In an unpredictable environment of drought, lightning, and additional biotic agents, the optimal proportion of untreated stressed trees cannot be determined.

Defoliators. Defoliators comprise the second-most-damaging insect group. The spruce budworm, Choristoneura fumiferana Clemens, is generally considered the most important forest defoliator in North America (Coulson

Neodiprion lecontei mating pair. Photo: G. Lintereur.
and Witter 1984, Morris 1963). Balsam fir, Abies balsamea L. Mill., is the
most preferred and susceptible species
(Mattson et al. 1983), although there
may be geographic variation in these
relationships. Populations normally
remain low for several decades, as
physical and biotic mortality agents
offset reproductive gains. Outbreaks
occur when there is an abundance of
mature host trees combined with sev-
eral consecutive years of hot, dry
summers (Blais 1973, MacLean
1980). Once populations rise, out-
breaks rapidly expand to most spruce
species.

Intense, continuous selection by re-
sistant trees would probably select for
immune C. fumiferana biotypes, just
as widespread DDT applications did
previously (Randall 1965). However,
behavioral and ecological attributes
could possibly be used to lessen the
chances of biotype formation against
this pest. If factors encoding for resis-
tance were incorporated into Picea
but not Abies, then a large proportion of
the population would complete de-
velopment on untreated plants. Peri-
odic outbreaks would continue to oc-
cur on the Abies refuge, but natural
enemies would eventually cause pop-
ulation collapse. Protection of the
more desirable Picea at the expense of
the less desirable Abies would proba-
bly be acceptable to forest managers,
based on the twofold difference in
their pulpwood values (Peterson
1988). Because young trees seem less
vulnerable to attack (Mattson 1985),
the possibility of limiting gene expres-
sion to older trees should also be
considered.

With such a scheme, genetically en-
gineered resistance could possibly be
integrated into current silvicultural
and biological controls. Because most
natural enemies of budworms do not
directly interact with the plant, for
example, there may be less exposure
to the novel trait than with bark
beetles. However, indirect effects re-
quire further investigation.

If efficacy were lost due to biotype
evolution, C. fumiferana populations
would not necessarily exceed their
current virulence. Host defensive
mechanisms mostly cause nonlethal
effects, such as smaller size and re-
duced fecundity, rather than direct
toxicity (Mattson et al. 1983). This
relationship could possibly reduce the
selective pressures for cross-resis-
tance, especially where silvicultural
practices provided species, age, and
variety mosaics.

One danger is that resistant trees,
although not directly affecting natural
enemies, would reduce their numbers
by depleting the supply of C. fumifer-
ana, and thereby allow a severe pest
resurgence once biotypes evolved.
Several factors, however, might re-
duce this danger. First, most of the
major predators and parasites that
maintain C. fumiferana populations
at low levels between outbreaks are
generalists (Morris 1963) that could
subsist on alternate insect hosts. Sec-
ond, the specialists that help termi-
nate outbreaks are adapted to long
periods of low C. fumiferana popula-
tion densities. Third, unlike tradi-
tional pesticide treatments that con-
tinually suppress natural enemies
while the resistant herbivore popula-
tion rises, a plant trait that does not
directly harm beneficial insects could
allow their populations to respond
immediately to increased prey densi-
ties. Effective emergency measures,
including a broad array of synthetic
and microbial insecticides, are avail-
able to prevent major losses if out-
breaks occurred despite the above
factors (Schmitt et al. 1984).

Although the concept of conferring
genetic resistance against C. fumifer-
ana merits further consideration, a
greater understanding of this insect’s
ecology and behavior is essential be-
fore deployment can be deemed rela-
tively safe. It is critical that bud-
worms feeding on Abies and Picea
do not become reproductively isolated.
The high dispersal ability and multi-
ple-oviposition behavior of the spruce
budworm lends some confidence to
this strategy, but additional research
should focus on gene flow and geo-
graphic variation (Hardy et al. 1983).

The specifics of the spruce bud-
worm–balsam fir–spruce system can-
not be generalized to all defoliators.
However, refugia may be maintained
for some other species by restricting
genetic alterations to certain growing
conditions. For example, the gypsy
moth, Lymnantria dispar L., causes
widespread forest defoliation, but
much of its economic damage occurs
in urban and suburban settings.
Therefore, a policy of deploying re-
sistant genes for ornamental or short-
rotation production purposes, while
practicing traditional and developing
integrated pest management strate-
gies in forests, could protect high-
value trees yet exert only moderate
selective pressures on the insect. Al-
though large acreages would still be
periodically defoliated, the actual
economic and aesthetic impact would
be greatly diminished. Again, the spe-
cific biologies of each insect must be
considered, and the inability of fe-
male gypsy moths to fly dictates cau-
tion (Table 2).

A similar strategy with novel genes
limited to short-rotation intensive
cultivations could be used against in-
sects such as the cottonwood leaf
beetle, Chrysomela scripta F., and the
forest tent caterpillar, Malacosoma
distria Hubner, which can cause
more severe economic losses in inten-
sive cultivations than in extensive for-
est.

Root insects. The impact of root-
feeding insects has been greatly in-
creased by modern forest plantation
practices (Schowalter 1985). Losses
to these species, primarily weevils
and white grubs, are generally low in
mature stands. However, when new
seedlings are established after harvest
or reclamation, root injury due to
larval feeding and/or adult stem gir-
dling can devastate plantings. Natural
enemies are valuable but inadequate,
and applied biological controls have
been unsuccessful. Likewise, there are
no totally acceptable silvicultural
remedies against species that feed on
living roots. Detection is difficult, so
soil-permeating, persistent pesticid-
es such as lindane are sometimes applied.
These chemicals have been banned for
most other uses. Thus, if transgenic
resistance could be employed against
root insects without adverse effects,
both tree production and environmen-
tal safety would benefit.

Root-feeding insects may provide
targets against which time-specific
expression of resistance genes could
reduce the chances of biotype evolu-
tion (Gould 1988, Raffa 1987). A bet-
ter understanding of the relationship
between host age and susceptibility is
necessary, however, to devise appro-
priate tactics. It is not known, for
example, whether losses are most se-
vere in young stands because larger
trees are more tolerant and can better
withstand feeding, if younger trees are more attractive or less able to resist attack, or if physical attributes of the soil created by a closed canopy simply reduce insect replacement rates.

If large trees are commonly exploited without suffering severe damage, then limiting resistance expression to young trees could provide refugia analogous to the fir trees left untreated for spruce budworms. If no such reservoir exists, biotype evolution would be likely. Moreover, more serious losses than currently occur could result. A synchronous large-scale surge of root insect populations rather than the current steady mortality to young trees would provide a vast substrate for species such as the pale weevil, Hylobius pales Herbst, that breed in dead tissue and then as adults girdle nearby live seedlings. If a substantial reservoir exists on mature trees but temporal relaxation of gene expression is not provided, the consequences could likewise be devastating. Increased root feeding on mature trees by adapted insects would increase susceptibility to bark beetles (Raffa 1988), which in turn could catapult scolytid populations across their threshold density and result in the massive outbreaks described previously.

Seed and cone predators. These species, primarily Lepidoptera, Coleoptera, and Hemiptera, pose major limitations on seed orchard productivity because their feeding translates directly into yield loss. They are only vulnerable to insecticides for brief periods of their life cycles, so timing is critical for effective control. Because multiple pest complexes are the norm, seed orchard managers must sample with an array of pheromones, consider numerous action thresholds, and spray for each population peak.

The use of resistant trees to suppress seed and cone pests could prove more compatible with biological control than current methods. With fewer insecticide applications, natural enemy populations of both target and currently sprayed nontarget species may rise, and multiple selection pressures could be enhanced. Regions outside seed orchards should be left unmanipulated. Usually seed orchards contain a large array of host lineages, thus providing an underlying genetic diversity that would further reduce unidirectional selective pressures.

The presence of neighboring untreated refugia is not sufficient to prevent biotype formation, however, as evidenced by numerous fruit orchard pestsw ith similar biologies that evolved insecticide resistance (Metcalf 1980). Untreated conebearing trees must be included within each planting. The greatest risks would probably be with species such as Conophthorus that enter cones as adults and oviposit in only one or several trees, rather than most Lepidoptera, which oviposit externally on the cones of many trees (Table 2). If biotypes arose in a seed orchard, there is no obvious mechanism by which neighboring forests would be threatened, as food scarcity would probably remain a major selective force in these areas.

Wood borers. These Coleoptera (Buprestidae, Cerambycidae), Lepidoptera (Cossidae, Aegeriidae), and Hymenoptera (Siricidae) primarily colonize weakened trees. Forests can be protected by early stand rotation, thinning, judicious site selection, and sanitation. In urban environments, however, numerous stresses such as root compaction, pollution, and mechanical damage render trees susceptible to wood borers. Thus, insects such as the bronze birch borer, Agrilus anxius Gory, are major pests of ornamental trees. Forest management strategies are not applicable to homeowners, and so insecticides are often required. These applications are expensive and only marginally effective, however, and insecticide drift poses major problems when large trees are sprayed in urban areas.

Widescale outplantings in commercial forests would pose some of the same risks as with bark beetles and are unnecessary. As with the gypsy moth, however, wood borers may provide targets where economic impact can be greatly reduced, with relatively low risk of biotype evolution, by limiting gene alterations to high-value trees.

Nontarget pest emergence and biotype-delaying tactics

In nature, all tissues of all tree species are exploited by a variety of insects.

Evaluating the influence of transgenic resistance on nontarget insects is extremely difficult. Because of their current nonpest status, we know the least about these insects. For most commercial tree species, the complete guild of insect herbivores has not even been cataloged (Niemela and Neuvonen 1983).

Although we have insufficient knowledge to predict nontarget pest emergence, experience with pesticides and resistant cultivars and observations from natural coevolved systems suggest some useful tactics. First, a mode of action that is relatively specific to the target organism and as distinct as possible from existing plant defense mechanisms should be selected. Although unpredictable forms of cross-resistance can occur, this approach would at least reduce the selection for coadaptive insect genes that provide immunity from both introduced and existing plant traits.

Second, expression of novel properties should be limited to the tissues and times at which the target feeds, and preferably expression should be induced by the target herbivore (Gould 1988, Raffa 1987). Naturally occurring tree-insect systems provide examples of how uneven phytochemical distribution can favor stability. Pines allocate resin acids to new but not old foliage, thereby protecting their photosynthetically most productive tissues while allowing herbivores to graze the less-valuable needles (Ikedada et al. 1977). Larvae that prefer the less-protected tissue presumably outcompete those not repelled by young needles, so physiological tolerance to resin acids has not evolved in most sawflies. Likewise, many plants limit the expression of defensive traits to critical periods of the growing season, certain age categories, or induction by herbivore activities.

The potential adverse effects of genetically engineered, whole-plant expression can be anticipated by a reexamination of the spruce budworm example, depicted earlier as a possibly safe target. If the defensive property inadvertently protected cortical tissue and thereby resisted spruce bark beetles, Dendroctonus rufipennis Kirby, then the processes described earlier as favoring bark beetle adaptations would operate. Although
this species is usually only a moderate problem, *D. rufipennis* can undergo outbreaks under favorable conditions, and it has caused some of the most severe losses ever recorded for any forest insect (Furniss and Carolin 1977).

Third, genotype mosaics should be employed within each planting, providing mixtures of transgenic traits superimposed on mixtures of various seed sources. Untreated trees should be intermingled within each planting. This strategy could reduce the likelihood of oligophagous and polyphagous herbivores emerging as new pests of formerly less-preferred trees by undergoing genetic shifts in behavior to avoid the novel defense (Gould 1984). Such genetic variation stabilizes potential outbreak species like the black pineleaf scale, *Nuculaspis californica* Coleman, in nature. Although this insect can develop within-tree biotypes that correspond to host genotypes (Edmunds and Alstad 1978), diversity within the host population provides a complex array of selective pressures that renders the adaptive value of various insect genes, such as those regulating movement and resource breadth, in opposition (Alstad and Edmunds 1983).

Fourth, we should adopt a major lesson from pesticide usage and devise comprehensive, preconceived biotype management programs (Brattsten et al. 1987). Insect monitoring is a critical component of this scheme. Both previous experience and theoretical models emphasize that tactics for suppressing biotype evolution are most effective before the newly favored allele becomes common (Brattsten et al. 1987, Tabashnik and Croft 1982). Both target and nontarget populations should be periodically appraised.

The population genetics of emerging biotypes should be thoroughly studied, as gene frequencies and inheritance patterns are critical in determining optimal suppression methods (Tabashnik and Croft 1982). Likewise, the mechanisms of biotype immunities should be characterized to help develop appropriate countermeasures (Brattsten et al. 1987).

### Developing strategies to minimize risks

The preceding analysis suggests specific tactics for estimating and reducing the risks of deleterious insect responses to transgenic trees. A scheme for integrating these actions in a cohesive fashion is proposed in Table 3. However, implementation of appropriate tactics will require a broad, interdisciplinary approach as an integral component of plant biotechnology. Several long-term strategies must be initiated.

First, specific guidelines on transgenic release that consider the chances of biotype evolution and nontarget pest outbreaks need to be established. Detailed, rigorous regulations already apply to laboratory and field practices with regard to human safety and/or accidental release (Brill 1985). Equivalent standards should be developed governing the impact of deliberate releases at the population and ecosystem levels. Replacing ad hoc decisions with established guidelines could also safeguard against the danger that each deployment resulting in no apparent and/or immediate adverse effects will lead to acceptance of other uses that are less judicious.

Policies must be based on research specifically directed at insect evolutionary responses to transgenic plants (Gould 1988). Research in this area is critically lacking. Laboratory models should be devised to test specific hypotheses, such as those emerging from Figure 1 and Table 2. Complementary studies on tree-insect interactions, insect population behavior and genetics, and community ecology should focus on nontarget pest emergence. Development of methods for restricting gene transfer (Bej et al. 1988) and limiting expression to specific tissues, times, and herbivore levels is a critical need requiring the skills of molecular biologists.

Heightened regulations always incur the risk of being counterproductive. Stipulations could become so restrictive as to render genetic alterations impractical and/or unattractive, thereby reducing the benefits this tool can bring to forestry. However, some of the systems where genetic engineering appears to have the highest margin of safety comprise large, well-defined, and easily accessible markets. Therefore, ecological and commercial considerations are often, or can be made, compatible.

The recommendation to limit expressed resistance to planted stock, for example, is of obvious benefit to biotechnology companies. Likewise, species such as *Salix* and *Populus*, which are most suitable for intensive short-rotation systems and gene mosaics, have also proven to be particularly amenable to protoplast manipulation and genetic engineering. Extension of patent life should also be considered as an incentive for accepting such guidelines.

The attributes of some potential target systems are likely to demand such expensive safeguards that deployment is not practical. This outcome is not justification for applying less-restrictive criteria, however, because the potential consequences of an erroneous decision are too severe. This philosophy does not discriminate solely against genetic engineering, but rather it is currently applied to such traditional tactics as importation of biological control agents.

Second, integrated risk management programs that involve all affected disciplines must be developed. Guidelines regulating gene transfers must be compatible with overall forest resource management. For example, wildlife biologists may oppose a

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**Table 3. Principal ecological and management strategies for reducing the risks and impact of biotype evolution associated with deployment of genetically engineered tree resistance.**

<table>
<thead>
<tr>
<th>Restriction of novel gene deployment to systems where multiple, opposing, and ephemeral selective pressures can be maintained</th>
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<tbody>
<tr>
<td>Implementation of biotype-delaying tactics at planting</td>
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<tr>
<td>Mode of action of introduced trait distinct from existing defense mechanisms</td>
</tr>
<tr>
<td>Temporal and spatial limitations on and herbivore induction of resistance trait expression</td>
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<tr>
<td>Host genotype mosaics at multiple layers</td>
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<tr>
<td>Intermingling with untreated trees</td>
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<tr>
<td>Biotype management</td>
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<td>Monitoring of field populations</td>
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<tr>
<td>Genetic characterization: mode of inheritance, dominance</td>
</tr>
<tr>
<td>Physiological characterization of biotypes: mode of detoxification, development of biotype modality inhibitors</td>
</tr>
<tr>
<td>Integration with other forest protection practices and multiple forest resource uses</td>
</tr>
</tbody>
</table>

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*S. W. Croft, E. F. W. Furniss, D. A. Palmiter, and Michael T. Tabashnik.*
particular approach because of its effects on insectivorous birds or other components of the food web. Likewise, trees genetically engineered to resist foliar fungal pathogens could possibly inhibit endophytes that repel insects (Carroll 1988), thereby causing nontarget insect outbreaks.

Finally, ecologists and plant protection specialists should become more involved in the training of molecular biologists. Although a proposed plant protection tactic may comprise a major scientific advance at the molecular level, its implementation could entail a quite primitive approach from a population perspective. Recent advances from all levels of biological organization must be integrated to enhance the efficacy and environmental safety of pest management tools.

More exposure to population genetics, population dynamics, and crop protection should be provided in the core curriculum of students intending to conduct plant genetic engineering. A historical context of previous technological capabilities that outpaced ecological understanding, such as high-grading, calendar pesticide application, and total fire suppression, would help better prepare molecular biology students for the contributions they can make.

Conclusions
An approach has been developed for using the general principles of biotype evolution to generate specific estimates of risk with regard to genetic engineering in trees and to devise possible preventive tactics. The major criteria include the tree-cropping system, compatibility with other pest management techniques, and specific attributes of the target insect’s biology. This approach may apply to other forms of plant genetic engineering, as well as to other biotechnological approaches to controlling insects. Based on these analyses:

- In some systems, biotype evolution poses a severe threat. Possible adverse effects include both decreased efficacy and alteration of existing plant-insect relationships so as to worsen current conditions. In other cases, genetic engineering could be more compatible with biological control than are current insecticide treatments or it could be used to provide greater genetic diversity than traditional breeding methods.

- In general, the risks are greater in large forested expanses than in seed orchards, rapid rotation systems, and ornamental plantings.

- Expression of resistance should be nontransferrable to host progeny.

- Genetic mosaics involving spatial, temporal, and herbivore-induced within-tree variation, multiple sources of resistance, treatment mixtures, and refugia of untreated trees can reduce risk. Natural systems provide valuable examples of stable tissue-protection strategies, and these systems should be emulated as models for ecologically sound transgenic tactics.

- Integrated biotype management practices that provide multiplicity and conflicting selective pressures, coupled with well-planned monitoring of and response to insect biotype emergence, could reduce risk. Introduced traits should be based on narrow modes of action that are as distinct as possible from existing host defense mechanisms.

- Biotype evolution needs to be considered for all gene transfers, regardless of their intended function. Likewise, intended resistance against insects must accommodate equivalent concerns from other ecological disciplines. Comprehensive, multidisciplinary ecological criteria governing the release of genetically altered trees should be researched and instituted.

- Greater emphasis should be placed on the scope of biological variation, the history of pesticide use, ecological feedback, and crop-protection principles during the training of molecular biology students intending to develop transgenic plants.

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