POPLAR AND *Bt*

Fiber Farming with Insecticidal

Naturally regenerated forests are the primary source of timber, fiber, and fuel throughout much of the world today. In the United States, however, public outcry over increasing forest fragmentation and habitat loss is reducing timber harvests in many areas. As our demand for forest products exceeds supplies, reliance on international timber resources will escalate, exacerbating global deforestation and timber shortages. Agroforestry—growing trees in intensively managed fiber or fuel farms—will lessen our demands on forest ecosystems in other parts of the world. Applications of plant biotechnology for the genetic improvement of woody plants, as for agronomic crops, are becoming increasingly valuable tools. Clearly, planning for future demand is essential because of the long production cycle of trees.

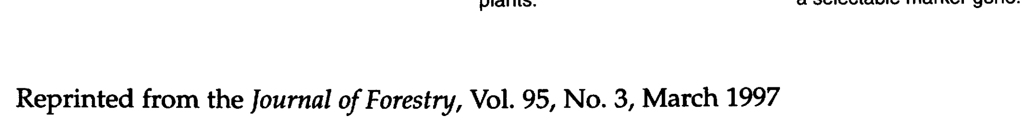
The intensive culture of short-rotation, hybrid *Populus* species is attractive because biomass and energy yields are high, agronomic technologies are well developed, and poplars are highly amenable to plant biotechnology. The inherent variability in *Populus*, coupled with advances in genetic engineering, will quicken the rate at which genetic improvements are made and facilitate their management.

The initial costs and practices of intensive tree culture are similar to those of other commodity crops: mechanization of site preparation and planting, fertilization, irrigation, and control of insects, weeds, and diseases. Although trees can withstand considerable damage and the economic injury threshold is high, costs associated with intensive culture could be reduced by the selection of clones with desirable attributes and genetic engineering. The first genetic transformation of hybrid poplar, in 1987, incorporated an herbicide resistance gene into the poplar genome, facilitating weed control in young plantations (Fillatti et al. 1987). Interest in improving pest resistance in poplars led to their transformation with a proteinase inhibitor gene cloned from potato (e.g., Chun et al. 1988) and a toxin gene cloned from *Bacillus thuringiensis* (McCown et al. 1991).

Bacillus thuringiensis (Bt) is an aerobic, spore-forming bacterium found throughout the world in insect-rich environments; it produces various crystalline proteins that when eaten are toxic to certain invertebrates. For more than 70 years, *Bt* strains were considered pathogenic only to caterpillars. Then, in 1977, a *Bt* strain that killed mosquito and black fly larvae was discovered (Goldberg and Margolit 1977), and in 1982 a different strain was found to kill beetle larvae (Krieg et al. 1983). These discoveries, as well as increasing restrictions on insecticides, mounting problems with resistant insects, escalating costs of insecticide development, and recent advances in plant genetic engineering, led to a virtual explosion in research on *Bt* diversity, genetics, physiology, structure, pathology, ecology, and toxicology (Pauls 1995). This effort continues and involves an international collaborative effort among researchers in biotech, agrochemical, pharmaceutical, seed industry, university, and government laboratories. At least 40,000 *Bt* isolates are now known, with pathogenicity among five additional insect orders and other invertebrate groups: mites, nematodes, flukes, mollusks, and protozoans (Feitelson et al. 1992; Bauer 1995).

The pathogenicity and narrow host specificity of *Bt* are controlled by protein toxins known collectively as

Engineering a transgenic insecticidal tree



Bacillus thuringiensis cell.

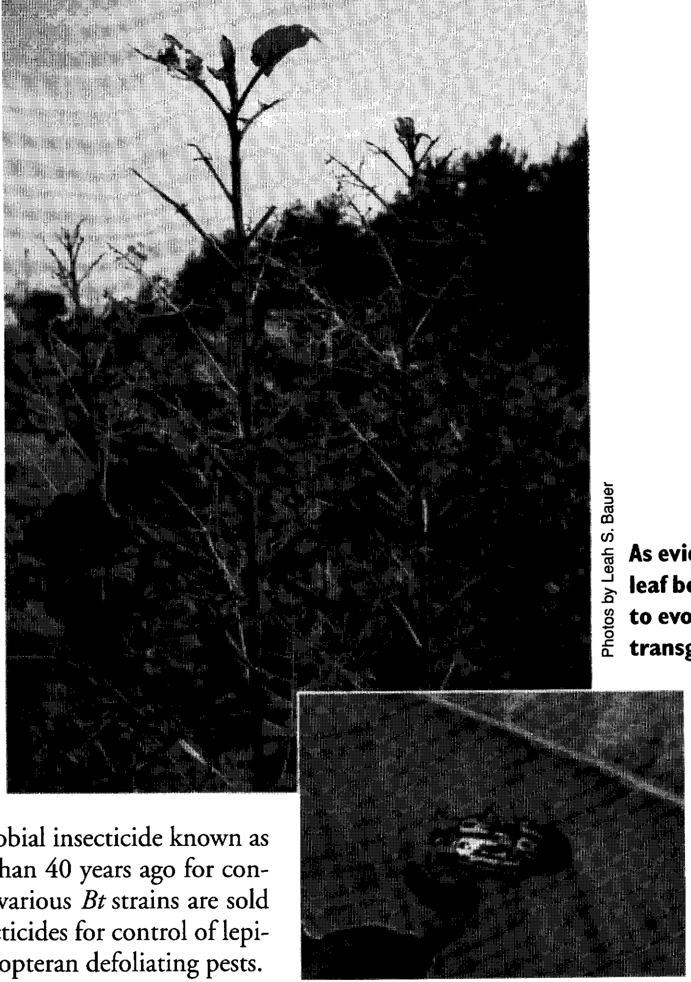
The *Bt* toxin gene is cloned and modified to improve expression, or synthesis, in plants.

The *Bt* gene is cloned into plasmid DNA of *Agrobacterium tumefaciens* along with a selectable marker gene.

By Leah S. Bauer

Reprinted from the *Journal of Forestry*, Vol. 95, No. 3, March 1997

Trees



Photos by Leah S. Bauer

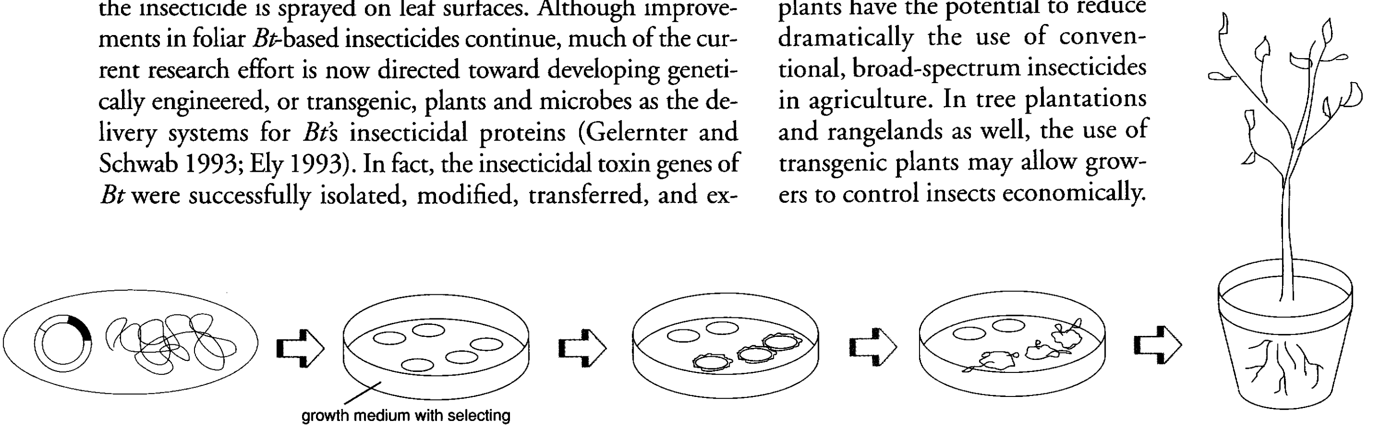
As evidence mounts that cottonwood leaf beetles have the genetic capacity to evolve resistance to *Bt* toxins in transgenic plants, operational strategies to conserve *Bt* susceptibility within populations of these defoliators will need to become increasingly sophisticated.

δ-endotoxins, Cry toxins, or insecticidal crystal proteins (e.g., Höfte and Whitely 1989). These crystalline toxins are produced abundantly during bacterial fermentation and, in conjunction with *Bt* spores, constitute the microbial insecticide known as *Bt*, commercialized more than 40 years ago for control of caterpillars. Today, various *Bt* strains are sold as safe, biodegradable insecticides for control of lepidopteran, dipteran, or coleopteran defoliating pests.

The specificity of *Bt* is highly desirable for control of insect pests in aquatic and forested ecosystems where conservation of other life forms, including beneficial and nontarget insects, is required. At present, the largest volumes of *Bt* are used to control forest defoliators. Despite *Bt*'s obvious attributes, it accounts for less than 1 percent of the global insecticide market because of its high cost and comparatively low efficacy. As concern for environmental quality and food safety increases, however, interest in *Bt* for managing agricultural pests and disease vectors has intensified.

One major impediment to more widespread use of *Bt* in agriculture is low efficacy resulting from UV degradation after the insecticide is sprayed on leaf surfaces. Although improvements in foliar *Bt*-based insecticides continue, much of the current research effort is now directed toward developing genetically engineered, or transgenic, plants and microbes as the delivery systems for *Bt*'s insecticidal proteins (Gelernter and Schwab 1993; Ely 1993). In fact, the insecticidal toxin genes of *Bt* were successfully isolated, modified, transferred, and expressed, or synthesized, in several target plants as early as 1987 (Kahl and Winter 1995). Five years later, at least 50 species of plants had been transformed with *Bt* toxin genes, and by 1996, *Bt*-transgenic cotton and potato were being grown commercially in the United States.

Transgenic plants containing toxin genes express the insecticidal proteins inside the plant cells, where the proteins are protected from UV and other forms of environmental degradation. Not only do transgenic plants deliver a relatively stable toxin dose to target pests, but also the *Bt* toxins can be expressed in specific plant tissues, providing control of stem-, root-, and fruit-feeding insects that could not be previously controlled by foliar insecticides. Unquestionably, transgenic plants have the potential to reduce dramatically the use of conventional, broad-spectrum insecticides in agriculture. In tree plantations and rangelands as well, the use of transgenic plants may allow growers to control insects economically.



Agrobacterium tumefaciens cell. (This bacterium normally causes crown gall disease, but its disease-causing gene has been eliminated.)

The *A. tumefaciens* cell infects leaf tissue, and foreign genes become randomly integrated into DNA of the host plant.

Calluses form on the leaf discs with the foreign genes. The selecting agent, or herbicide, in the growth medium prevents nontransformed leaf discs from regenerating.

Shoots develop from resistant leaf discs.

Shoots regenerate into trees that produce *Bt* toxin in some or all tissues. Each transformed tree is tested for insect toxicity and growth rate; good performers may then be cloned and field tested.

Transgenic Trees

In 1991 hybrid poplar became the first woody plant to receive a *Bt* toxin gene *(cry1A(a))* (McCown et al. 1991). The *Bt*-transgenic trees had greater resistance to defoliating caterpillars (Robison et al. 1994). Insecticidal poplar clones transformed with the *Bt cry1A(c)* gene, which is also toxic to caterpillars, are now under large-scale field evaluation in six provinces in China (Wang et al. 1996). The discovery that the Cry3A protein from *Bt* var. *tenebrionis* was toxic to the cottonwood leaf beetle *(Chrysomela scripta)* led to the registration of a microbial insecticide to control this destructive pest of young poplar plantations (Bauer 1990; Bauer and Pankratz 1992). This finding also led to current research on poplar transformation with the *cry3A* gene in several laboratories throughout the world.

Our initial enthusiasm for *Bt*-transgenic plants, however, is now tempered by the knowledge that insects have the genetic capacity to evolve resistance to *Bt* toxins. Resistance to *Bt* is reported from laboratory selection experiments in nine species of Lepidoptera and two species each of Diptera and Coleoptera, including the cottonwood leaf beetle (Bauer et al. 1994). Furthermore, several populations of the diamondback moth have developed resistance in different parts of the world after intensive management with foliar applications of *Bt* in the field (Tabashnik 1994a).

Before those reports, the notion that insects could become resistant to these toxins had been greeted with considerable skepticism because *Bt*, unlike conventional insecticides, had remained efficacious after several decades of commercial use. It is now believed that the lack of field resistance in the past resulted from low selection pressure exerted by early formulations and usage patterns (Stone et al. 1991).

Managing Resistance

The high-dose, single-toxin strategy in the current design of transgenic plants will ultimately select for resistance in insect populations because the capacity for resistance exists within the genome of all insect species (Denholm and Rowland 1992). We are now faced with the challenges of designing and manipulating operational strategies that conserve *Bt* susceptibility within the pest population. Resistance management strategies were recently reviewed in some detail by McGaughey and Whalon (1992), Whalon and McGaughey (1993), Tabashnik (1994a,b), Bauer (1995), and Wearing and Hokkanen (1995). Although not yet validated through long-term field studies, models suggest resistance can be delayed by various means:

1. *Mixtures* of toxins with different mechanisms, either within the same plant or in different plants, or expressed serially over time;
2. *Synergists* to increase toxicity;
3. *Rotations* to alternative toxins to reduce the frequency of resistant individuals;
4. *Refuges*, both temporal and spatial, to facilitate survival of susceptible individuals;
5. *Low doses* of toxin that produce sublethal effects, such as reduced egg production and slowed development, thereby increasing the insects' vulnerability to predators, parasites, pathogens, and other mortality factors;

6. *Ultrahigh doses* of toxin to kill individuals that contain resistance genes;

7. *Gene regulation* of toxin concentration, location, and induction to avoid constant exposure in all plant parts.

The objective is to minimize the exposure of the target pests to the toxins in time and space—a challenging task when one is dealing with such long-lived, perennial plants as trees. Nevertheless, the high economic damage thresholds and long rotation of woody plants should encourage plant geneticists and growers to incorporate resistance management strategies into the design and deployment of transgenic woody plants containing toxin genes.

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