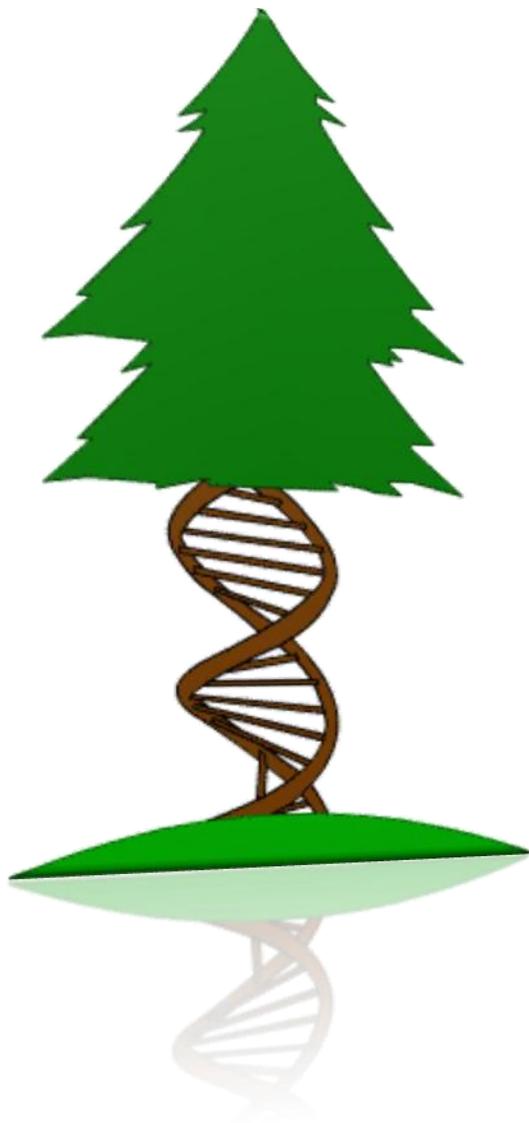


# Genetic Modification in Forestry: Methods and Reasons



Alistair Beck

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## **Abstract**

Rising demand for lumber and mounting economic pressure are increasing the need for a more cost efficient and reliable method of growing timber. The use of transgenic methods can allow for greater survival and growth rates in trees. Several different methods of genetic modification can be employed including biological and non-biological techniques. The reasons for modification can include the introduction of resistance to both biotic and abiotic disturbances or the improvement of wood characteristics. Although there are many benefits that can come from genetically modifying trees, there are some potential problems that may arise, including resistance to foreign genes or cross contaminating natural trees with those modified genes.

## **Keywords:**

Forestry, transgenic, genetic modification, modification methods, GM Deletor, GM Containment

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## Introduction

Throughout history, humans have manipulated their surroundings to enhance their way of life. An example of this manipulation is the domestication of plants and animals which in turn led to the altering of these organisms through the use of selective breeding. In the past few decades scientists have been able to isolate specific DNA sequences that code for the genes that control how an organism develops. Methods have also been developed for specific gene sequences from one organism to be transferred to another biologically unrelated organism. Any organism that has had its genome altered is referred to as a transgenic or genetically modified organism (GMO). In past decades there has been a great deal of research aimed at genetically altering crop plants, while in recent years many researchers have shifted their focus to trees. This increased focus has led to new techniques and methods of genetic modification and greater understanding of tree genetics. This paper will examine several of these techniques and types of genetic modification along with the potential risks that transgenic trees create.

## Methods of Recombinant DNA Creation

In 1973 the first transgenic organism was created when an *Escherichia Coli* bacterium was modified to express a salmonella gene. (Cohen et al., 1973) This genetically modified organism (GMO) was created using DNA that was artificially created. This is known as

recombinant DNA. Several different methods of recombinant DNA creation are used in the production of GMOs. The two most common of these methods are known as bacterial and non-bacterial transformation (Kuure-Kinsey & McCooey, 2000). These transformation techniques do not transfer recombinant DNA directly to plant cells but are an important middle step.

## **Biological Transformation**

Of the two main modification methods, biological transformation is traditionally more commonly used. This is due to the now relative ease with which biological transformations are conducted. Bacteria will accept foreign DNA from naked DNA sources, such as plasmids and phages under certain laboratory circumstances, including heating and chemical introduction (Roe, 2007). Within the biological branch of recombinant DNA creation there are two common methods known as bacterial transformation and phage introduction.

## **Bacterial Transformation**

Bacterial transformation is defined as the exchange of genetic material between strains of bacteria by the transfer of a fragment of naked DNA from a donor cell to a recipient cell, followed by recombination in the recipient chromosome (Elsevier, 2007). The recombinant DNA creation method known as bacterial transformation was the original means of transferring genetic material from one organism to another and is still the most popular method used today. The initial trials were conducted between two different strains of *E. coli* bacteria. Each

strain was adapted to thrive in a specific nutrient solution. When placed in the incompatible solution the bacteria did not grow. When the two strains were placed together and allowed to intermingle it was discovered that genes for survival in the opposite solution was passed between opposing bacteria. This transfer of genes allowed the bacteria to thrive in solutions which were originally unsuited for their existence. It was observed that the bacteria needed to make physical contact with each other for the transfer to occur (Griffiths et al. 2000). With this discovery researchers realized that it was possible to transfer DNA sequences between different organisms.

The portion of the bacterial DNA that is transferred is found in the plasmid, an extra-chromosomal DNA molecule independent from the bacterium's chromosomal DNA. Plasmids are similar in some ways to viruses, in that they are not classified as a form of life by current standards and that they have the ability to self-replicate within proper host cells. However plasmids differ from viruses in several ways, including their biological make up. Plasmids consist of naked DNA, where viruses do not and plasmids lack the genes necessary for independent transfer to other cells. (Kuure-Kinsey & McCooey, 2000) The lack of histone proteins on naked DNA allow for a much simpler transfer between cells than that of regular DNA, which needs to have its histone proteins dissolved before the double helix structure separates and allows for DNA transfer. The purpose of plasmids in bacterial cells is to provide resistance to antibacterial toxins or alternately to allow for the production of antifungal toxins (Kimball's Biology Pages, 2008). It is believed that plasmids arose in response to the competition between bacteria, molds and fungi.

The presence of plasmids within bacterial lifeforms can be utilized as a tool to transfer DNA fragments between organisms. Geneticists are able to take snippets of DNA from a foreign organism and use the rapid reproduction rate of bacteria to create many duplicates.

## Phage Introduction

Phage introduction is a method of recombinant DNA creation that is similar in many ways to bacterial transformation. Rather than using bacterial plasmids to transfer DNA fragments the phage introduction method uses bacteriophages, more commonly known as phages. Phages are viruses that attack bacteria by injecting their DNA into the bacteria where it will use the bacteria's biological resources for the replication of phage DNA. There are two infection cycles, lytic and lysogenic that can occur after a phage has infected a bacteria cell and in most cases the infection is lethal to the bacteria. After a bacterium is infected the phage DNA will take over the biological processes of the bacteria and make copies of itself until the bacterial resources are depleted, or the quantities of replicated DNA are too large for the bacteria to contain and its cell wall bursts.

Unlike the structure of a bacteria cell, which has organelles for energy production and waste removal, viruses only contain the naked DNA required for replication enclosed in a protein envelope (Todar, 2008). The viral structure is exceptionally suitable to aid in the creation of recombinant DNA, where rather than taking numerous steps to transfer DNA between several different bacterial cells, a phage's DNA can be replaced with the desired foreign DNA and then be allowed to attack the transferring bacterial cell.

## Non-Biological Transformation

Unlike biological transformation techniques, non-biological transformation methods do by definition not use biological agents to transfer DNA. Non-biological transformation methods force foreign DNA into cells by using chemical, physical or enzymatic treatments. These methods skip the initial bacterial step in the gene transfer process and insert the DNA straight into the cell without the intermediary. These techniques usually have a higher mortality rate of host cells than those that are biological in nature.

## Recombinant DNA Transfer to Plant Material

Some of the techniques for transferring genetic material to bacterial cells can also be used for transference to the plant cell. However not all transfers can be accomplished with these techniques and new ones needed to be developed.

## Biological Transfer

After a segment of foreign DNA has been transferred to a bacterial cell, the DNA needs to be conveyed to the final recipient. There are a few biological transfer methods, but the most commonly used method is the use of a plant attacking bacteria genus known as *Agrobacterium*. The use of biological transfer methods usually has a higher survival rate of host cells in the transfer process.

## Agrobacterium

The genus *Agrobacterium* within the bacterial kingdom, is a Gram-negative bacterium which in nature causes tumors in plants by using horizontal gene transfer. *Agrobacteria* are known to naturally transfer genetic information, for gall formation, into the host plant's genome. The DNA that is transferred is found within the plasmid rather than the bacterial chromosomal DNA. Even if the DNA responsible for gall formation is removed and replaced with a foreign DNA sequence, the *Agrobacteria* is still able to transfer the DNA to the host plant. Since *Agrobacteria* are able to transfer DNA sequences to different species, they are known as a "transport vehicle" for the propagation of new genes. (Saftey, GMO, 2006)

The process of gene transfer requires several steps, starting with the creation of binary vectors. The genes, necessary for horizontal gene transfer in the *Agrobacteria*, are known as virulence genes and to prevent uncontrolled spreading of the introduced DNA, need to be removed for the initial stage. The foreign DNA is placed between the borders where the virulence genes were located, replacing the original gall forming gene sequence. The removed virulence genes are placed in a second plasmid, thereby allowing the *Agrobacteria* to still transfer genes but only those that have been chosen to be inserted. The first vector is propagated in *E. coli* and then transferred to an *Agrobacteria* using either the bacterial transformation or phage introduction technique. After the two plasmids have been reunited in a single *Agrobacteria*, it is allowed to attack plant cells, thereby transferring the inserted genes to the plant cells.

## Non Biological Transfer

All living cells have a membrane surrounding them. This functions as a screen to prevent the incursion and retreat of certain molecules into and out of the cell. This outer layer, known as the plasma membrane, consists of a semi-permeable lipid bilayer. The plasma membrane is made up of two layers of phospholipid molecules which arrange themselves with their hydrophobic (water fearing) “tails” facing inwards and their hydrophilic (water loving) “heads” facing outward. Due to the polar nature of this arrangement many molecules cannot enter or exit the cell without the use of specialized protein molecules. Unfortunately for researchers, DNA molecules are one of the particles that cannot freely enter the cell through the plasma membrane and since most research techniques require DNA from a foreign source to be inserted into a cell a method of insertion needed to be developed. The three most commonly used techniques involve the use of chemicals, electricity, and biolistic particle delivery systems.

## Chemical Transfer

Due to the chemical makeup of organic cells, certain specific chemical solutions can aid in the uptake of DNA. The main chemical used in the chemical transfer process of foreign DNA to cells is calcium chloride. The chloride ions within the solution are able to permeate cell membranes, while the calcium ions are unable to penetrate. As the chloride ions move through the cell membrane they bring positively charged water molecules along. This increase in water causes the cells to swell and heat, which in turn opens pores in the plasma membrane and

allows for DNA penetration. To aid the process the solution containing the cells and calcium chloride is heated.

### **Electroporation**

Electroporation is a method of DNA transfer that uses an electrical current to manipulate the plasma membrane. Due to the nature of the phospholipid bilayer, it can be disrupted by an electric current passed through a cell. For this technique to properly function, the plant cells and DNA strands need to be suspended in solution. Once the solution is created, a device that generates an electrical charge is inserted into the solution. The solution is subjected to a short pulse of electricity, usually lasting for a few microseconds to a millisecond. During the time that the electrical charge is active, small aqueous pores open along the phospholipid bilayer and the polarity of the interior of the cell changes. Due to the change in polarity and the aqueous pores, DNA molecules are driven into the cell. Since there are no chemical bonds that connect the bilayers of the plasma membrane, no permanent damage occurs from the electrical charge. After the electrical charge has ended the hydrophobic and hydrophilic interactions reinitiate thereby allowing the plasma membrane to reform (McCord, 2003).

### **Particle Bombardment**

Another non-biological transfer method is known as particle bombardment. Here plant tissue is bombarded with particles of heavy metals coated in foreign DNA. In most cases the plant material that is the target of the gene transfer are undifferentiated plant cells known as

callus. These callus cells are grown in similar conditions as bacterial cultures, usually in an agar plate, where they can survive indefinitely as long as nutrients are available. Callus cells on an intact plant are the cells that grow on the surface of a wound to protect them from bacterial and fungal attacks (University of Liverpool, n.a., n.d.).

The process of particle bombardment is carried out by a piece of equipment referred to as a gene gun or biolistic particle delivery system. This transfer method uses microscopic heavy metal particles, usually gold or silver, coated in plasmid DNA to transfer genes to plant host cells (Hain & Ehly, 2000). The DNA coated heavy metal particles are physically projected at a Petri dish containing callus where a high proportion of cells are destroyed. A percentage of the cells that survive the bombardment will have had the DNA coated heavy metal particle hit the nucleus of the cell, causing the foreign DNA to be absorbed by the plant chromosome. When the callus cells are removed from their agar plate and allowed to differentiate, they will pass on the introduced gene to their daughter cells, thus growing a plant with the desired foreign genes.

## **Gene Containment**

### **Ablation**

This method of gene containment uses promoters in specific cells to control the production of a deleterious gene (Brunner, et al., 2007). This gene usually codes for a

cytotoxin, which when introduced into the reproductive cells of the tree causes sterility. Although the cytotoxins are only meant to be produced in the reproductive cells of the trees occasionally the genes are expressed in other parts of the tree as well. When cells in other parts of the tree are also affected by the genetic manipulation and begin to express the gene for cytotoxin production, the tree's overall growth may be detrimentally affected. The drawback to this method of gene containment is that no fruits will be produced, resulting in a decrease of food for many forest dwelling animals. Also, this method is not wholly efficient, with some trees still being able to reproduce in spite of the cytotoxin.

### **Gene Excision (Gene Deletor)**

The gene excision or gene deletor technique of gene containment uses site specific recombinase systems to remove transgenic material from cells before reproduction occurs (Brunner, et al., 2007). Along with adding the genes that produce the desired attributes in the gene excision process, a gene is added that will remove the transgenic genes from the DNA strand during meiosis. Since the process of mitosis only occurs in the reproductive centers of a tree, the transgenic material is only removed when sex cells are being produced. Given that ordinary cell replication occurs through the process of mitosis and not meiosis, the gene deletor does not remove the transgenic material and the daughter cells produced in the process still contain the inserted genes. Although this process is similar to the "terminator" technology there are some advantages to the gene deletor technique. The gene deletor technique allows for functioning fruit, where the "terminator" technique does not (Li, 2007). This technique

allows for trees to be protected from insects and/or abiotic pressures while still permitting natural tree regeneration and still supply a food source for forest dwelling animals.

## **Gene Suppression**

The gene suppression technique is similar to the ablation method in that it prevents trees from reproducing rather than removing the transgenic material. Unlike the ablation technique gene suppression does not add a toxin producing gene, but rather it disables the natural reproduction cycle. This disruption can be achieved in several different ways including RNA interference, dominant negative proteins and repressors of flowering. In the RNA interference (RNAi) method of gene suppression, RNA is used to interfere with the production of reproductive proteins. The RNAi attaches to the protein producing RNA strand, inhibiting the access of amino acids to the strand, thereby blocking protein production. This method of gene suppression has been found to be robust and reliable, since redundancies can be built into the RNAi suppression. Another method of gene suppression is the production of dominant negative proteins which make a specific portion of the reproductive cycle repressive, thereby disabling it. One example of this is the repression of floral development, which in turn makes natural reproduction unlikely. The gene suppression technique has potential to achieve gene containment but in turn reduces the amount of food for animals.

## **Gene Repression**

Repression of flowering is an alternative that postpones reproduction, rather than disabling it. This method can be utilized by postponing reproduction past the age of harvest, so

that the trees will be cut down long before they are ready to reproduce. Once again the gene repression technique allows for gene containment but animals are affected by the lack of fruit production.

## **Reasons for Tree Modification**

### **Insect Resistance**

There are many challenges, including biotic and abiotic disturbances, which trees face within the different ecosystems that they inhabit. Many losses in the forest industry are due to natural disturbances such as wind, fire and insect/fungal attacks. In past decades the control of fire damage has been a large priority of governments with fire fighting techniques constantly being improved upon. Methods for the prevention of wind damage have also been implemented. Branches are trimmed and forest edges are feathered to form a lower surface area for wind to catch. These methods of controlling fire and wind damage are now common and effective. The prevention of insect and fungal attacks are however not as effective as they could be. With the current methods of insect control being limited to the placement of partially effective traps, or wide scope pesticide spraying which does not have complete coverage and is damaging to the environment, the options for a forest manager are quite restricted.

Much of the transgenic forest research has been focused on the implementation of insect damage prevention. One of the most effective systems has been the introduction of gene sequences from *Bacillus thuringiensis* (BT); a soil bacterium with insecticidal toxins, into

the genomes of trees. Although BT insecticides have been used as aerosols for years, their effectiveness is still limited to coverage and dispersal patterns. Not only are the aerosol versions of the BT insecticides ineffective, they can also cause environmental damage by killing non-pest insects in the area. Using transgenic techniques, it is possible to transfer the gene sequence that codes for toxin production from the BT bacterium into the genome of trees. After the insertion of this gene sequence the trees will produce the toxin themselves and no longer need to be sprayed with pesticides.

## **Environmental Resistance**

There are many environmental factors that can detract from the growth potential of trees in the wild. Of these environmental factors, there are several that the focus on research into the introduction of genes to improve survivability. These research areas include improved frost hardiness, improved levels of soil salinity tolerance, and drought resistance.

An improved level of frost hardiness would allow trees to survive long cold winters or sudden cold fronts that can occasionally hit forest trees. There are occasions where trees are damaged in early spring when buds are awakening and are frozen by a cold spell this affects the growth potential for that growing season. The use of genes from more frost hardy species would allow transgenic trees to survive these cold fronts with little to no damage, greatly improving their growth for the upcoming year. An increased level of soil salinity tolerance would allow for improved afforestation potential in areas with high soil salinity. There are many regions that have a soil salinity level that is too high for most species of trees to grow. If

genes from plants that have a higher tolerance for elevated levels of soil salinity are introduced into the genome of trees, they will be able to thrive in highly saline soils. An improved level of drought resistance would also allow for improved afforestation potential and help to stem the spread of deserts in drier regions. Genes from desert plants can be extracted and inserted into the genomes of trees so that afforestation projects can become successful in dry regions.

## **Wood Manipulation**

Although there has been much research into protecting trees from natural disturbances such as environmental and insect damage, the manipulation of the wood properties themselves has also been researched. Several different avenues of research have been conducted with regards to wood quality. Improved fibre content and strength along with reduced levels of lignin are several of the adaptations that will allow transgenic trees to play a greater part in the fibre market.

## **Ecological Effects and Possible Remedies**

When modifying the genome of organisms, there are many risks involved. Not only can modification be improperly performed, but there are ecological consequences of genetically modified (GM) trees interacting with the natural environment. These interactions can include a loss of natural species from over competition by GM species, gene flow from GM species to natural species, and disruption of the food web.

## Competition

Since many of the modifications being applied to trees are for improved tolerance against biotic and abiotic disturbances, there is a clear competitive advantage that is gained over their natural counterparts. Thus if some transgenic trees were able to escape into the wild, they might be able to thrive and may eventually out compete the natural trees. One of the possible solutions for this problem in species that are shade intolerant is to include a gene for dwarfism in the modification process. The offspring of the trees would eventually be unable to grow in the natural environment due to their inability to survive in the shadows of other trees being exasperated by the dwarfism gene.

## Gene Flow

Since many of the trees that are being genetically modified are native to their area, there is a very high risk that they will transfer transgenic material to the surrounding natural populations. This transfer could be potentially devastating to the natural balance of ecosystems and could eventually cause natural species to become extinct. The possible solution for the prevention of gene flow between transgenic and natural trees is to implement GM deleter genes in the original genetic modification.

## Food Web Damage

With one of the main focuses of transgenic forestry being the protection of trees from insect damage and infestations, there are implications that need to be taken into consideration. Since the current strategy for defending trees from insects is for the trees to create their own toxin and thus killing the insects, there is the potential for increased strain on already pressured food chains. Many forest dwelling birds, small mammals, and reptiles rely on insects for a substantial proportion of their nutritional intake. With transgenic trees that kill these insects, the lower levels of the food web are adversely affected. One solution to this would be to genetically alter trees to become undesirable to insects, rather than kill them.

## Global Transgenic Forestry

Forest GM Activities of the World

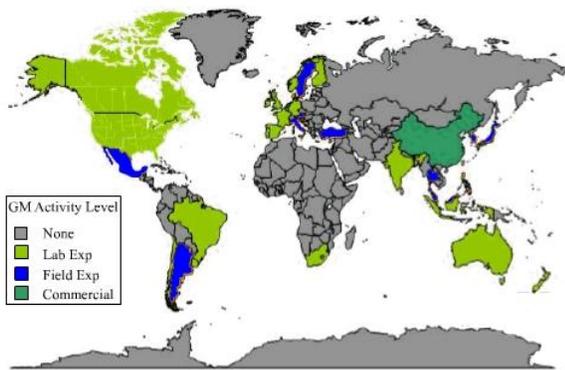


Figure 2: Global GM Forest Activity (FAO Forestry Department, 2004)

There are many countries in the world that are currently undertaking transgenic forestry research and due to the regulations in these countries, all modified specimens need to be contained to prevent ecosystem contamination. Several of the countries conducting research, including Canada, have legislated that only laboratory experiments may be performed, while

a few countries have authorized field experiments. As of 2004 China is the only country that has sanctioned commercial transgenic forestry activities (FAO Forestry Department, 2004). As more countries begin field testing of transgenic trees, there is a greater potential for a breach in

containment of test facilities thereby allowing transgenic material to escape. To combat this possibility many researchers are working on techniques for gene containment.

## Current Research

### Genetics of Resistance to Transgenic *Bacillus thuringiensis* Poplars in *Chrysomela tremulae* (Coleoptera: Chrysomelidae)

Augustin et al.

Augustin et al. (2004) focuses on the introduction of *Bacillus thuringiensis* (Bt) genes into a *Populus tremula* L. x *Populus tremuloides* Michx clone and the possibility of increased resistance of the Poplar Leaf-Beetle (*Chrysomela tremulae*) to the toxin. The hypothesis is that due to the increase in toxicity in the foliage of the poplar trees, there will be a corresponding increase in resistant to Poplar Leaf-Beetles. A lab experiment was conducted to determine if there were any resistant beetles, and if so, if the traits were dominant or recessive and if they would be passed down to the offspring. Resistant beetles were identified by feeding groups of beetles with either transgenic leaves or normal leaves. Any beetles that survived the transgenic leaves had their genes examined to determine which allele was responsible for the resistance. The allele frequency for resistance in the experiment between the years of 1999 – 2001 was determined to be 0.0037 (Augustin et al., 2004). The next experiment was to determine if the allele for resistance was conferred by a single autosomal gene. This was accomplished by having different strengths of the toxin. The experimenters then crossed a group of resistant

males with a group of susceptible females, and then crossed the offspring with each other to create a mixed population known as F<sub>2</sub>. The F<sub>2</sub> group was then once again isolated into groups, with one group fed with non-transgenic leaves and the other group was fed on transgenic leaves. The group eating the non-transgenic trees only suffered a 16.67% mortality rate. When attempting to estimate the Lethal Concentration where 50% of the population is killed (LC<sub>50</sub>) for the resistant strain, the researchers discovered that the beetles did not die from eating the leaves and that they could not create a high enough concentration of the toxin to kill enough of the beetles. The highest mortality rate they could achieve was 18%. This indicates that the resistant strain's LC<sub>50</sub> is 6410 times that of the susceptible. The experiments proved that the resistance rates from the first generation hybrid strand and the resistant strand indicated that the dominance rate of the resistance allele is lower than 0.07 which therefore proves that the resistance is almost completely recessive. The resistance ratios were calculated by dividing the LC<sub>50</sub> for the strain or cross in question by the LC<sub>50</sub> for the susceptible strain. This research showed that there can be beetles that are resistant to the toxin created by the BT protein but the allele that gives the resistance is extremely recessive and therefore quite rare and difficult to pass down to the beetle's offspring.

### **The cry3Aa gene of *Bacillus thuringiensis* Bt886 Encodes a toxin against long-horned beetles**

Chen, J et al.

Chen et al (2005) reveal that they have found a strain of *Bacillus thuringiensis* (BT) whose toxin specifically targets long-horned beetles. The toxin only differs from the previously

discovered strain by six nucleotides and four amino acids, but has a 75% mortality rate against long-horned beetles. The long-horn beetles cause damage to almost 2.7 million ha of forests in over 30 countries. This equates to an estimated cost of \$4 billion USD in China alone (Chen et al. 2005). In an attempt to control the insects, many steps have been taken. Chemical and biological insecticides, as well as integrated management have been attempted. All of these attempts have proved ineffective due to the health hazards and financial commitments (Chen et al. 2005). Due to the failure of the other treatments, some Chinese scientists have suggested using trees that create their own toxins and thereby kill defoliators right at the source. Transgenic trees that use the BT toxins eliminate the need for insecticides as well as inhibiting the larval invasion of larvae into the cambium and xylem.

The first experiment was a bioassay of the toxin. Larvae were given a feed that contained a ratio of 10:1 toxin solution for two months. After the two-month period the surviving larvae were weighed. The mortality rate was 60% and the surviving beetles had an average weight that was approximately 62% lower than normal beetles. With this discovery, the researchers have proposed that the toxin-producing genes could be introduced into the cambium, thereby killing or inhibiting growth of larvae. This proposition is different than most others in this area, where most modification takes place in the leaves of the trees.

### **Field evaluation of insect-resistant transgenic *Populus nigra* trees**

Hu, J.J. et al.

The most important tree in the Chinese plan to create a defensive perimeter around the desert that was created when the natural forest was obliterated, is the poplar. The reason for

its importance is its high growth rate, easy propagation and a wide range of usage (Hu et al. 2001). The poplar is considered to be the most important tree species for establishing artificial forests and plantations. One major drawback is that they are susceptible to defoliators. In 1989 over 10,000 hectares, approximately 40% of the total poplar plantation area in China, was defoliated (Hu, Tian, Han, Li, & Zhang, 2001). As a result of the large percentage of defoliator attacks, transgenic poplars have been field-tested. It was found that the trees had almost complete protection against the gypsy moth and forest tent caterpillar. In the spring of 1994, poplar trees, 151 from one line expressing the BT gene, and 52 from two lines of non-transformed were planted in one plantation. Another non-transformed plantation was planted 2 km away. A total of 100 leaves were sampled from each tree and categorized into five classes 100% damaged, 50% damaged, 25% damaged, less than 25% and undamaged. The average damaged leaf rate in the transgenic trees was 10% while the non-transgenic trees that were planted 2 km away had a rate of 80%. The damage to the first line of non-transgenic trees in the same plantation was 11% and the second line was 7%. These results indicate that transgenic trees not only have resistance to insects but also affect the non-transgenic trees in the vicinity. A pupa survey was also preformed, where thirty plots of 1 m x 1m x 0.2 m were randomly selected in both the transgenic sites as well as non-transgenic sites. The number of pupa found in the non transgenic plantation soils were between 4.9 – 4.1 times higher than in the soil of the transgenic plantation. Due to the protection of the transgenic trees the other trees in the plantation did not have to be sprayed with pesticides. Unlike the non-mixed plantation the pure non-transgenic plantation was sprayed but still suffered much damage. Although the

researchers are optimistic about the application of transgenic trees in plantations and even in natural forests, they state that long-term studies of the transgenic trees should be continued.

## **Conclusion**

With the ever increasing number of methods of genetic transfer, it is becoming more financially viable for companies and governments to implement transgenic trees. Before genetically modified trees can be used they must lose the social stigma that they carry, which can only be done by proper education. With all of the potential benefits that transgenic trees promise there is still much research that is required before commercial plantations can be safely implemented within natural ecosystems.

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