GENETIC ENGINEERING OF SEXUAL STERILITY IN SHADE TREES

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Abstract. Shade trees unable to produce floral tissues, or that produce only nonreproductive floral organs such as petals, are desirable for a number of reasons. They can reduce the need to clean flower and fruit litter, eliminate hazards from large and fleshy fruits on walks, and lessen allergenic pollen production. Research in herbaceous species has established that introduction of gene constructs created by recombinant DNA technology provides an effective means to manipulate flowers without deleterious effects on vegetative growth. Though not yet demonstrated in trees, this approach will likely be successful in both angiosperms and gymnosperms because genes that control reproductive development are similar in sequence and function among diverse plant species. Key to the practical application of genetically engineered sterility to shade trees, however, is the development of efficient gene transfer and vegetative propagation systems to deliver engineered, sterile trees to the marketplace; these systems are in place for a limited number of species. We discuss the rationale for sexual sterility in arboriculture, methods for genetic engineering of sterility, our progress in engineering sterility in poplars, and the current status of transformation and propagation methods for some common shade tree genera.

Keywords. Flowering; fruit trees; gene transfer; clonal propagation; transgenes; floral homeotic genes.

Genetic transformation is the introduction of new genes, referred to as transgenes, via nonsexual processes. The modified host, a transgenic organism, typically expresses this new transgene and thus possesses a new trait. The entire process of gene isolation, modification, and transfer to a new organism is known as genetic engineering.

Transformation of plants was first accomplished using tobacco in 1984. In the 13 years since then, over 120 species in at least 35 families have been transformed (Birch 1997). In the United States, over 3,500 field trials of transgenic plants are in progress or completed (USDA-APHIS 1997). Furthermore, 29 transgenic crops have been commercially released or approved for release as of December 1997. Transgenes introduced into crops include those conferring resistance to insects, viruses, or herbicides; male sterility; and a wide variety of quality traits such as modified fruit ripening. Genetic engineering is clearly no longer just a scientific research tool; it is making rapid inroads into all areas of agriculture.

Not unexpectedly, the genetic engineering of trees lags well behind that of herbaceous crops. However, substantial progress has been made with several genera. Eight different genera are represented in field tests of transgenic trees, including apple (Malus), plum (Prunus), sweetgum (Liquidambar), and walnut (Juglans) (USDA-APHIS 1997). Furthermore, the commercial potential for genetically engineered trees in commercial tree clones has been clearly demonstrated in poplars (Populus). Introduction of genes conferring resistance to the herbicide glyphosate produced striking results in both greenhouse and field studies (Strauss et al. 1996, 1997). All nontransgenic control trees sprayed with herbicide (Roundup Pro™) were severely damaged, while a large proportion of transgenic lines showed complete or near complete tolerance to the herbicide. Initial results with transgenic poplars expressing a Bacillus thuringiensis toxin gene indicate a high level of resistance to the major pest of poplars, the cottonwood leaf beetle (Strauss et al. 1997). In addition to growth and management traits, wood quality traits, particularly modification of lignin content, are also major areas of research.

Of interest to many arborists is reproductive sterility, a quality trait that has already been successfully introduced into herbaceous species via transformation. The objectives of this paper are to describe the rationale for genetically engineered sterility in shade trees and the methods available to accomplish it. In addition, we cite examples of our progress in engineering sterility in poplars to illustrate the potential, and state-of-the-art of this technology, for trees.
Rationale for Sterility
Though varying in degree among species, fruit litter is often a substantial annoyance (Barker 1986). For example, sweetgum is extensively planted due to desirable traits such as fall leaf color, but the tree's spiny fruits disintegrate very slowly and cause a nuisance on lawns and walks. The fleshy fruits of cherries, plums, and apples often create slippery surfaces and adhere to feet, resulting in floor stains. Senescing fruit of many trees is undesirable to shopkeepers and homeowners because these trees attract insects and disease and cause unpleasant odors. Floral litter, such as from catkins, often passes through screens and contributes to clogging of drains. Though fruit litter can be limited by using only male trees of dioecious species or fruitless cultivars, many desirable shade trees are neither dioecious nor known to have sterile clones (Barker 1986). Finally, elimination of pollen is desirable because many people suffer from allergies induced by tree pollen, an effect which is likely to be exacerbated by fertile trees planted close to homes.

By constraining sexual propagation, sterility can provide several advantages. It would restrict thefts of proprietary germ plasm to vegetative propagules, which can be more easily identified than seedlings by DNA fingerprinting and other methods. Sterile cultivars are more highly contained from unrestricted spread via pollen and seeds, thus greatly minimizing the chances for novel or engineered varieties to escape and become a nuisance in wild or managed environments. This feature is likely to be important to winning regulatory approval for marketing of transgenic varieties of trees (Strauss et al. 1995).

Genetic Engineering of Sterility
The first steps in the process of genetic engineering are the isolation and manipulation of genes before introduction into a plant (Figure 1, step 1; see Table 1 for definition of terms). Usually, a cDNA rather than a gene is used as a transgene. When a gene is expressed, RNA is transcribed from one strand of DNA. Subsequently, the RNA undergoes modification, including the removal of introns, to produce messenger RNA (mRNA), which is translated into protein. cDNAs can be produced in the laboratory from mRNA isolated from living cells; because they lack introns and are thus smaller than complete genes, they are more easily manipulated (Figure 1, step 2). A promoter is a regulatory DNA sequence that directs the transcription of a gene and is located upstream of the gene it controls, though additional regulatory sequences may be present at other sites. Promoters may be constitutive, directing expression of their genes in virtually all tissues at all times, or they may cause activation only at certain times and/or in certain cell types. Promoters from different kinds of genes can be readily swapped using recombinant DNA methods, providing many options for controlling the expression of transgenes (Figure 1, step 3).

Generation and commercial application of sterile trees requires reliable transformation and propagation systems. This includes a procedure to deliver genes into cells so that the genes may become stably incorporated into the genome (Figure 1, steps 4–5); either Agrobacterium-mediated
transformation or bombardment with DNA-coated microprojectiles usually serves as the gene trans-
fer agent (Birch 1997; De Block 1993). Other key
steps include differentiation of transformed cells
with active genes from nontransformed cells (Fig-
ure 1, steps 6–7), regeneration of transgenic cells
into plants (step 8), molecular methods to verify
that the transgene is present in the plant’s ge-
nome and that mRNA is produced (step 9), the
ability to vegetatively (or sexually) propagate a
transgenic plant for testing and use (step 10), and
verification of its value and delivery of the new
trait (step 11).

Woody plants have generally been considered
recalcitrant to transformation. However, reliable
transformation systems have recently been de-
veloped for several difficult agronomic and woody
species. For example, cereals were once con-
sidered highly difficult to transform, but rice is now
routinely transformed, and at least 8 transgenic
varieties of maize are in, or near to, commercial
use. Efficient transformation systems can be de-
veloped for most trees given sufficient effort, as
demonstrated by recent advances in transformation of
poplars, apples, eucalyptus (Eucalyptus), sweetgum,
Prunus, and pines (Pinus) (Table 2).

Sterility methods. Engi-
neered sterility results from
either ablation (cell death) of
floral tissues, or modification
of floral organs due to inhib-
ited expression of genes es-
sential for reproductive
development. Ablation
methods use a promoter
that is active only in floral tis-
sues to regulate the expres-
sion of a gene encoding a
cytotoxin. Because the cy-
totoxin is produced only in
floral cells and cannot pen-
trate a cell membrane, flou-
ral tissue is destroyed while
vegetative tissues are una-
fected. In many cases, a
RNase (enzyme that degrades RNA) serves as
the cytotoxic gene.

Strategies for inhibiting gene expression act
at 1 of 3 levels. Either transcription of the gene is
blocked, the mRNA is not translated into protein,
or the activity of the encoded protein is inhibited.
Usually, a transgene incorporated into a plant’s
genome is expressed. However, when a promoter
or transgene homologous to an native gene is
introduced, a proportion of the regenerated trans-
genomic plants exhibit gene silencing, also referred
to as sense suppression or cosuppression (Flavell
1994; Matzke and Matzke 1995). Expression of
both the transgene and endogenous gene is sup-
pressed. In some cases, transcription of the
transgene and endogenous gene is inhibited,
while in other cases the transcribed mRNAs are
degraded before they are translated into protein.

Antisense-suppression is a related phenom-
emon that acts by either reducing mRNA transla-
tion or by increasing mRNA degradation (Mol et
al. 1994). A cDNA is placed under the control of a
promoter but in an opposite orientation to that of
Table 1. Glossary of common terms in genetic engineering.

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition/Explanation</th>
</tr>
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<tbody>
<tr>
<td>Ablation</td>
<td>Elimination of undesired tissues by causing cell death</td>
</tr>
<tr>
<td>Agrobacterium</td>
<td>A bacterium that transfers DNA into plant cells; used for genetic engineering of plants</td>
</tr>
<tr>
<td>Antisense-suppression</td>
<td>A method for inhibiting a gene’s expression by introducing a reverse copy of the gene</td>
</tr>
<tr>
<td>cDNA</td>
<td>Synthesized from a mRNA in vitro; corresponds to the expressed form of a gene (without introns)</td>
</tr>
<tr>
<td>Constitutive gene expression</td>
<td>A gene expressed in virtually all cells at all times</td>
</tr>
<tr>
<td>Cytotoxin</td>
<td>A protein that, when expressed in specific cells, causes cell death</td>
</tr>
<tr>
<td>Dominant negative mutation (DNM)</td>
<td>A mutation in a gene that results in a nonfunctional protein that also inhibits the activity of the wild-type protein</td>
</tr>
<tr>
<td>Homeotic gene</td>
<td>A gene that when rendered nonfunctional causes an organ to develop in the wrong place (e.g., petals develop where stamens normally form)</td>
</tr>
<tr>
<td>Inflorescence</td>
<td>Structure on which flowers occur (e.g., a catkin)</td>
</tr>
<tr>
<td>Intron</td>
<td>DNA sequence that interrupts the protein-coding sequence of a gene; present in nearly all genes in higher organisms</td>
</tr>
<tr>
<td>Gene expression</td>
<td>The process by which genetic information is read and turned into mRNA and then protein</td>
</tr>
<tr>
<td>Gene silencing</td>
<td>Inhibition of a gene’s expression</td>
</tr>
<tr>
<td>Genome</td>
<td>All the DNA sequence contained in an organism</td>
</tr>
<tr>
<td>Meristem</td>
<td>Groups of undifferentiated cells from which organ-forming cells arise (e.g., within apical buds and cambium)</td>
</tr>
<tr>
<td>Messenger RNA(mRNA)</td>
<td>Complimentary to one strand of DNA, after introns are removed; serves as the template for synthesizing a protein</td>
</tr>
<tr>
<td>Promoter</td>
<td>A regulatory DNA sequence located in front of the coding portion of a gene; it controls when, where, and to what level a gene is expressed</td>
</tr>
<tr>
<td>Sense-suppression</td>
<td>A method for inhibiting a gene’s expression by introducing a duplicate or slightly mutated version of the gene</td>
</tr>
<tr>
<td>Sequence conservation</td>
<td>The sequence of a particular gene is similar across diverse species; sequence of a gene isolated in one species can be used to easily isolate the corresponding gene (homolog) from a different species</td>
</tr>
<tr>
<td>Transcription</td>
<td>The synthesis of RNA from DNA</td>
</tr>
<tr>
<td>Transcription factor</td>
<td>A protein that regulates the expression of genes by interacting with their promoters</td>
</tr>
<tr>
<td>Transgene</td>
<td>A gene introduced into the chromosome of a plant via a nonsexual process</td>
</tr>
<tr>
<td>Translation</td>
<td>The synthesis of a protein from a mRNA template</td>
</tr>
<tr>
<td>Wild-type gene</td>
<td>A &quot;normal&quot; gene that encodes a fully functional protein</td>
</tr>
</tbody>
</table>

the native gene. As a result, the wrong DNA strand is transcribed, resulting in an antisense mRNA that is not translatable into protein and that is complementary to, and thus inhibits, translation of the endogenous sense mRNA. Gene silencing may be a result of activation of natural systems for cellular defense against aberrant genes and viruses (Ratcliff et al. 1997); however, the mechanisms are not fully understood. Finally, reversion to a nonsuppressed state has been observed in some cases (e.g., Jorgensen 1995), and suppression is often partial, with some gene expression remaining. It is therefore important to test transgenic plants produced by this method thoroughly to ensure the trait is stable.

The final strategy employs transgenes with dominant negative mutations (DNMs). A gene with a DNM encodes a mutant protein that is not only nonfunctional but also inhibits the activity of the coexisting, wild-type protein (Herskowitz 1987). The sequence of a cDNA is altered in vitro to generate a DNM, placed under the control of a strong promoter, and introduced into a plant. Though not yet extensively studied in plants, many DNMs are potent inhibitors of wild-type function in other eukaryotic organisms. The modular structure of regulatory proteins, such as those encoded by floral homeotic genes (described below), makes them particularly useful for generating DNMs.

**Floral homeotic genes.** To engineer sterility by these methods, promoters of genes expressed only in floral tissues are necessary for the ablation approach, while cDNAs of genes essential for reproductive development are required for the suppression strategies. Floral homeotic genes and their promoters fulfill both of these requirements. Furthermore, their high level of DNA se-
Table 2. State of transformation, regenerability, and propagation of common shade tree genera.

<table>
<thead>
<tr>
<th>Genus (common name)</th>
<th>Regenerability</th>
<th>Propagation</th>
<th>Transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abies (fir)</td>
<td>0, E (Mohan et al. 1995b)</td>
<td>V, NM</td>
<td></td>
</tr>
<tr>
<td>Acer (maple)</td>
<td>0, E (Grahsl et al. 1991)</td>
<td>V, M</td>
<td></td>
</tr>
<tr>
<td>Crataegus (hawthorn)</td>
<td>0, E (Mohan et al. 1995a)</td>
<td>V, M</td>
<td>Macrae and Van-Staden 1993; Teuliéres et al. 1994</td>
</tr>
<tr>
<td>Eucalyptus (eucalyptus)</td>
<td>0, E (Mohan et al. 1995a)</td>
<td>V, M</td>
<td></td>
</tr>
<tr>
<td>Fraxinus (ash)</td>
<td>0, E (Mohan et al. 1995a)</td>
<td>V, M</td>
<td>Huang 1993; Huang et al. 1991; Shin et al. 1994</td>
</tr>
<tr>
<td>Larix (larch)</td>
<td>0, E (Mohan et al. 1995a)</td>
<td>V, M</td>
<td>Chen and Stomp 1991; Sullivan and Legramini 1993</td>
</tr>
<tr>
<td>Liquidambar (sweetgum)</td>
<td>0, E (Bajaj 1989)</td>
<td>V, M</td>
<td>Wilde and Merkle, 1994</td>
</tr>
<tr>
<td>Malus (apple, crabapple)</td>
<td>0, E (Mohan et al. 1995a)</td>
<td>V, M</td>
<td></td>
</tr>
<tr>
<td>Picea (spruce)</td>
<td>0, E (Mohan et al. 1995b)</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>Pinus (pine)</td>
<td>0, E (Mohan et al. 1995b)</td>
<td>V, NM</td>
<td>Walter et al. 1997</td>
</tr>
<tr>
<td>Pistacia (pistache)</td>
<td>E (Onay et al. 1995)</td>
<td>V, NM</td>
<td></td>
</tr>
<tr>
<td>Platanus (plane tree, sycamore)</td>
<td>O (Bajaj 1991)</td>
<td>V, NM</td>
<td></td>
</tr>
<tr>
<td>Poplar (cottonwood, aspen)</td>
<td>O, E (Mohan et al. 1995a)</td>
<td>V, M</td>
<td>Han et al. 1996</td>
</tr>
<tr>
<td>Pseudotsuga (Douglas-fir)</td>
<td>O, E (Mohan et al. 1995b)</td>
<td>V, M</td>
<td></td>
</tr>
<tr>
<td>Quercus (oak)</td>
<td>E (Mohan et al. 1995a)</td>
<td>V, NM</td>
<td></td>
</tr>
<tr>
<td>Rhododendron (rhododendron, azalea)</td>
<td>O, E (Bajaj 1989)</td>
<td>V, M</td>
<td></td>
</tr>
<tr>
<td>Sequoia (Sierra redwood)</td>
<td>O, E (Mohan et al. 1995b)</td>
<td>V, M</td>
<td></td>
</tr>
<tr>
<td>Sequoiodendron (coast redwood)</td>
<td>O (Mohan et al. 1995b)</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>Ulmus (elm)</td>
<td>O, E (Bajaj 1989; Mohan et al. 1995a)</td>
<td>V, M</td>
<td>Sticklen et al. 1994</td>
</tr>
</tbody>
</table>


*Key references are listed for genera that have been transformed.

*Abbreviations: O, organogenesis; E, embryogenesis; V, vegetative propagation; M, micropropagation; NM, no commercial micropropagation.

*Only partial or limited success.

Floral homeotic genes encode transcription factors that control floral development and have been especially well-studied in the model herbaceous species *Arabidopsis thaliana* (a member of the mustard family) and *Antirrhinum majus* (a member of the snapdragon family). Homologs of genes cloned in these 2 species have been isolated and characterized in dicots, monocots, conifers, and ferns, indicating that floral homeotic genes have fundamental roles in reproductive development of all land plants. Studied genes fall into 2 broad functional classes: those controlling meristem identity and organ identity.

Floral meristem identity genes mediate the transition from an inflorescence meristem to a floral meristem (Figure 2a). The *Arabidopsis* genes LEAFY (LFY) and APETALA1 (AP1) are initially expressed throughout the floral meristem, and mutations in these genes cause a transformation of flowers into inflorescence shoots (Weigel and Meyerowitz 1994; Yanofsky 1995). Additional genes have been identified that play at least minor roles in specifying floral meristem identity.

Floral organ identity genes are necessary for 3 different homeotic functions, designated A, B and C, which specify the 4 different organ types present in most angiosperms (Figure 2). Each of these activities functions in 2 adjacent whorls: A activity specifies sepals in whorl 1, combined AB activities specify petals in whorl 2, BC activities specify stamens in whorl 3, and C activity specifies carpels in whorl 4. AP1 and APETALA2 are A function genes, APETALA3 (AP3) and PISTILLATA are B genes, and AGAMOUS (AG) is the only known C gene (Weigel and Meyerowitz 1994; Yanofsky 1995). These genes are expressed before the pri-
A. VM -» IM

B. Whorl: Floral Meristem Identity Genes

Figure 2. Model for how floral homeotic genes control flower development. (A) Before a plant is able to flower, its vegetative meristem must undergo a transition to an inflorescence meristem. Next, the floral meristem identity genes initiate the formation of floral meristems on the sides of the inflorescence meristem. The floral organ identity genes then direct the differentiation of floral organs from the floral meristem. VM, vegetative meristem; IM, inflorescence meristem; FM, floral meristem. Panel (B) shows the floral organ identity model (Coen and Meyerowitz 1991). The floral meristem of the typical angiosperm is divided into 4 concentric rings or whorls. Each whorl gives rise to a different floral organ. The combinatorial action of 3 classes of floral organ identity genes, designated A, B, and C, determines what floral organ develops and in which whorl the organ develops. Each class of genes functions in 2 adjacent whorls (indicated by the rectangles).

Figure 2 shows the model for how floral homeotic genes control flower development. (A) Before a plant is able to flower, its vegetative meristem must undergo a transition to an inflorescence meristem. Next, the floral meristem identity genes initiate the formation of floral meristems on the sides of the inflorescence meristem. The floral organ identity genes then direct the differentiation of floral organs from the floral meristem. VM, vegetative meristem; IM, inflorescence meristem; FM, floral meristem. Panel (B) shows the floral organ identity model (Coen and Meyerowitz 1991). The floral meristem of the typical angiosperm is divided into 4 concentric rings or whorls. Each whorl gives rise to a different floral organ. The combinatorial action of 3 classes of floral organ identity genes, designated A, B, and C, determines what floral organ develops and in which whorl the organ develops. Each class of genes functions in 2 adjacent whorls (indicated by the rectangles).

mordia of the organs they specify emerge from the floral meristem, and organs in 2 adjacent whorls are transformed (e.g., petals are replaced by sepals) if one of these genes is nonfunctional.

AP3 is expressed only in cells giving rise to petals and stamens. When the AP3 promoter was fused to a cytotoxic gene and introduced into tobacco or Arabidopsis, petals and stamens failed to develop, resulting in a flower consisting of 1 whorl of sepals and 1 whorl of carpels (Day et al. 1995). Because AP1 is initially expressed throughout the floral meristem shortly after the meristem begins to form (Gustafson-Brown et al. 1994), introduction of an AP1 promoter-cytotoxin construct may completely ablate all floral organs. In contrast, the expression pattern of AG predicts that an AG promoter-cytotoxin construct will ablate stamens and carpels but not sepal and petals. Promoters from nonfloral homeotic genes have also been used to engineer sterility (e.g., Mariani et al. 1990; Goldman et al. 1994). However, these genes are expressed at the last stages of flower development, and promoter-cytotoxin constructs typically prevent either the formation of viable pollen or prevent fertilization of carpels but not both. Thus, these promoters are less versatile than homeotic promoters for engineering a completely sterile plant.

Suppression of floral homeotic genes can also result in bi- or unisexual sterility, as well as generate novel kinds of flowers. Inhibition of AG activity by either DNM or antisense approaches produced completely sterile flowers with a sepal-petal-petal pattern (Mizukami and Ma 1995; Mizukami et al. 1996). Due to AG's additional role in floral meristem determinacy, this pattern was repeated several times, resulting in particularly attractive flowers. This strategy might be useful for improving attractiveness of Rosaceous tree species (e.g., Prunus, Malus), while eliminating development of their fleshy fruits. In AP3 mutants, sepals and carpels develop normally, but sepals develop in place of petals in the second whorl and carpels develop in place of stamens in the third whorl, resulting in a male-sterile flower with a sepal-sepal-carpel-carpel pattern. When both LFY and AP1 are inactive, all flowers are transformed into completely sterile inflorescence shoots (Weigel and Meyerowitz 1994; Yanofsky 1995).

**Early flowering.** The prolonged juvenile phase of trees is a major obstacle to evaluation of recombinant gene constructs for sterility. In some species, such as apple and eucalyptus, chemical and physical treatments that induce precocious and heavy flowering are used routinely. Though similar methods may be effective in additional species, at least 1 to 3 years are still likely to be required before flowering. Recently, an additional approach that uses floral homeotic genes was demonstrated. Several genes that are normally involved in floral initiation and development can induce precocious flowering when constitutively expressed (Nilsson and Weigel 1997). When the Arabidopsis gene LFY was constitutively expressed in aspen (P. tremula × P. tremuloides), flowering occurred within months
Progress Engineering Sterility in Poplars

Because floral homeotic genes are substantially conserved among species, it is possible that heterologous genes and promoters can be used to engineer sterility in poplars and other trees. However, there are significant risks with this approach. The distinct morphology and development of poplar flowers compared to that of all the well-studied herbaceous species suggests that important differences in gene expression and function will occur. Promoters and genes from another tree with similar morphology, particularly one belonging to the same family, are more likely to work predictably.

For these reasons, we have chosen to focus on poplar floral homeotic cDNAs and promoters for use in engineering sterility. We have isolated 4 cDNAs and genomic clones (genes and promoters) from Populus trichocarpa (black cottonwood) that are homologous to genes well-characterized in Arabidopsis and Antirrhinum (Table 3). Not unexpectedly, we have detected differences in expression and function between the poplar genes and herbaceous homologs. Based on the expression patterns of the poplar genes as well as the function of homologs, we are generating constructs for engineering sterility.

We are using these cDNAs and the floral-specific promoters to engineer sterility via promoter-cytotoxin, antisense, and DNM approaches. Because inhibition of multiple homeotic genes may yield more complete and stable sterility than single gene inhibition, we plan to also produce constructs designed to inhibit 2 or more genes. For the ablation approach, we have examined various tissues for gene expression and are us-

Table 3. Summary of 4 poplar floral homeotic genes. (+) = gene expression detected; (−) = gene expression not detected; (+++) = gene expression detected at a higher level.

<table>
<thead>
<tr>
<th>Poplar gene</th>
<th>Expression in developing flowers</th>
<th>Vegetative expression</th>
<th>Arabidopsis homolog</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTFL</td>
<td>++</td>
<td>++</td>
<td>LFY</td>
</tr>
<tr>
<td>PTD</td>
<td>+</td>
<td>++</td>
<td>AP3</td>
</tr>
<tr>
<td>PTAG1</td>
<td>++</td>
<td>++</td>
<td>AG</td>
</tr>
<tr>
<td>PTAG2</td>
<td>++</td>
<td>++</td>
<td>AG</td>
</tr>
</tbody>
</table>
ing promoters of genes for which only floral expression has been detected. However, whether a promoter will direct stringently floral-specific expression of a cytotoxin through many growing seasons and environments is uncertain. Use of an endogenous promoter also risks gene silencing, so that the cytotoxin is not expressed. Finally, the function of poplar genes may differ significantly from homologs in model species, so suppression of these genes does not produce the desired result. By empirically testing a number of sterility constructs, we expect to identify at least a few that result in stable and useful sterility even if these problems occur.

To expedite analysis of sterility constructs, we are also investigating ways to produce early-flowering poplars (Strauss et al. 1996). Five heterologous genes shown to induce early flowering when constitutively expressed are being analyzed in transgenic poplars. To date only the 

\[ LFY \]

transgene has caused precocious flowering as previously reported (Weigel and Nilsson 1995), and only in specific genotypes of male poplars (Figure 3). However, aberrant carpel-like structures have been occasionally observed in female transgenic poplars. Surprisingly, constitutive expression of the poplar \n
\[ LTFL \]

homolog, \n
\[ PTFL, \]

has not induced early flowering after 3 years (\n
\[ LFY \]

induced flowering within months). This suggests that poplars have evolved mechanisms different from those present in \n
\[ Arabidopsis \]

control the initiation of flowering.

**Summary**

We reviewed the rationale and methods for genetic engineering of sterility in trees. Advances in the molecular biology of flowering in herbaceous species show that genetic engineering of sterility in shade trees is feasible. Engineered sterility can relieve the substantial problems of fruit litter and pollen production, facilitate regulatory approval for transgenic trees, and obviate the need for flower control via application of growth-regulating chemicals. Sterility that affects one or both sexes, and that can impair the reproductive organs (stamens, carpels) while preserving or enhancing floral display organs (perianth), can be accomplished with current technology. Gene transfer and propagation methods exist for many genera to a sufficient degree to enable successful application of this technology. Among common temperate shade trees, methods are most advanced for \n
\[ Malus, Populus, Prunus, Liquidambar, \]

and \n
\[ Eucalyptus. \]

Progress in isolation, configuration, and testing of floral genes from trees is most advanced in poplars and will provide a model and source of transgenes for genetic engineering of other woody species.

**Literature Cited**


\[ Prunus \]


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Résumé. Les arbres ornementaux incapables de produire des tissus floraux ou qui ne produisent que des organes floraux non reproducteurs sont recherchés pour diverses raisons. Ils peuvent réduire les besoins en ramassage de fleurs tombées, éliminer les risques d'accidents provoqués par de gros fruits charnus sur les trottoirs, diminuer les besoins en ressources chez les arbres en stress physiologique, et diminuer la production en pollen allergène. La recherche chez les espèces herbacées a permis de prouver clairement que la technologie d'introduction de gènes reconstruits, grâce une recombinaison de l'ADN, fournit un excellent moyen pour manipuler les structures florales sans effet secondaire sur la croissance végétative. Même si ce n'est pas encore démontré pour les arbres, cette approche semble être promettante à la fois chez les angiospermes et les gymnospermes parce que les gènes contrôlant le développement reproductif sont conservés parmi les diverses espèces de plantes. La clé pour l'application de la stérilité génétique aux arbres ornementaux est le développement de systèmes de propagation végétative et de manipulation efficace pour fournir des arbres stériles sur le marché. On y discute de la rationalité d'un stérilité sexuelle en arboriculture, des méthodes de stérilité en génie génétique, des progrès pour rendre stériles les peupliers et de l'état d'avancement des méthodes de propagation et de manipulation chez certains genres communs d'arbres.


Resumen. Los árboles de sombra que no producen tejidos florales, o que producen solamente órganos florales reproductivos, son deseables por un número de razones. Ellos reducen la necesidad de barrer sus flores, eliminan los peligros de grandes y carnosos frutos sobre las aceras, reducen el drenaje de recursos de árboles fisiológicamente estresados y aminoran la producción de polen alergeno. La investigación con especies herbáceas tiene claramente establecido que la introduccion de genes creados por la tecnología de recombinación de DNA provee un medio efectivo para manipular las estructuras florales sin deteriorar el crecimiento vegetativo. Aunque no ha sido aún demostrado en los árboles, esta aproximación es probable de ser exitosa en angiospermas y gimnospermas, debido a que los genes que controlan el desarrollo reproductivo están conservados entre diversas especies de plantas. La clave para la aplicación práctica de la ingeniería genética de la esterilidad para los árboles de sombra es el desarrollo de eficientes sistemas de propagación vegetativa y transformación para llevar árboles estériles al mercado. Discutimos la razón de la esterilidad sexual en arboricultura, los métodos para la ingeniería genética de la esterilidad, nuestros avances en ingeniería de la esterilidad en álamos, y el estado actual de la transformación y métodos de propagación para algunos géneros comunes de árboles de sombra.