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THE BIOTECHNOLOGY OF URBAN TREES[1]

by Brent H. McCown

Abstract. Trees are some of the most difficult plants to manipulate in breeding and improvement programs. Plant biotechnology offers a unique set of tools that allows the plant breeder heretofore unprecedented ability to precisely change and clone such woody perennials. Although the future of biotechnology of trees is revolutionary, there are some major problems that must be solved before the full potential of this technology can be applied to urban tree improvement. Nevertheless, major advances in genetic transformation, cloning, and regeneration of trees have been recorded in the last few years; continued progress will depend on the effort applied to tree biotechnology and to determining the physiological/genetic bases of urban tree growth and ecology.

In many parts of the United States, this 1988 summer has surely been the year that we need some magic wand to help in growing urban trees. Record heat and drought conditions have combined with all the other stresses that urban trees must overcome to produce innumerable dying or unthrifty specimens. Although I would not be so naive to suggest that biotechnology is this magic wand, I will document that this technology has given us tools that will contribute immensely to our ability to provide trees with heretofore unprecedented qualities.

Plant biotechnology can be considered to consist of two rather distinct but interrelated processes: *in vitro* plant cloning or 'micropropagation' and manipulation of genes or 'genetic engineering'. Although both of these aspects of biotechnology began rather isolated from each other, with the recent commercialization of this science, we have seen a merging and complementation of the two.

Woody perennial plants such as trees have been the last crops to which biotechnology has been applied. This is in part due to the lack of a defined interest group promoting work on these plants and in part due to the perceived difficulties in working with these types of plants as compared to herbaceous crops. Recently, however, limited but important progress has been made with trees; in this paper it is my goal to discuss this progress and to indicate where biotechnology may or may not be revolutionary for trees. I will restrict my comments to angiosperm trees useful for urban plantings.

Tree Micropropagation

Street and ornamental trees standardly have been propagated by seeds or by asexual (clonal) methods. As new selections have become more popular, cloning, usually by budding onto seedling rootstocks, has become the most common method of street tree propagation. Thus arborists are familiar with the advantages and disadvantages of clones.

With other horticultural crops such as ornamental herbaceous plants, shrubs, and small fruits, cloning by use of 'test-tube' or *in vitro* techniques (micropropagation) has become an important alternative cloning method (11). Micropropagation has found wide use for a variety of reasons:

1. Micropropagation may be the only practical method of cloning a plant in large numbers. Examples include *Kalmia* (mountain laurel), some northern blueberries, some nut species (walnut), and some tropical fruits (palms and bananas).
2. A crop initially propagated by micropropagation may produce plants of superior form or uniformity. This has particularly been the case

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for indoor ornamental plants.

3. Because of its sterile source, micropropagated stock is more readily kept disease-free during multiplication and thus such problems as virus contamination can be controlled. This aspect is of importance for such crops as fruits (eg. strawberries) and vegetables (eg. potatoes and mint).
4. Although other commerical cloning methods may exist, micropropagation may be used to very rapidly increase the stock available. This stock can them be used to rapidly enter the market or to generate stock plants that will form the basis for cloning using standard cuttings.

For street and ornamental trees, micropropagation has not been heavily utilized. Since nurseries have had such methods as budding available, retooling for a new method that deals with small, succulent plants usually shipped by a speciality micropropagation laboratory has not seemed desirable. In addition, methods employing propagation by cuttings have advanced for some trees (eg. red maples, crabs) and thus have further reduced the desire or need to resort to alternative methods such as micropropagation. However, the major reason why more urban trees have not been micropropagated is that attempts at cloning these plants using *in vitro* methods have often not been successful. In order to understand some of the problems that are being encountered, a quick summary of the micropropagation process is necessary.

Most all the micropropagation processes now in commercial use involve shoot cultures, and thus only this process will be discussed here. Shoot cultures are masses of rapidly-growing shoots which usually have resulted from the stimulation of axillary buds on the subcultured shoot (branching) (Figure 1). If properly done, such cultures can be indefinitely maintained *in vitro* by sequential subculturing of shoots onto fresh medium and the subsequent stimulation of axillary development. For harvest, fully developed shoots are removed from culture and treated as very small cuttings ('microcuttings').

Micropropagation can be considered to consist of three phases (8). In the first, the isolation phase, tissue is isolated from the stock plants and placed in culture under aseptic conditions. Thus this phase involves both the sterilization of the plant tissue and stimulation of the initial shoot growth. Although complications such as internal bacterial contamination can be a problem, this phase is usually not the major limitation to micropropagation of trees.

The second phase is critical and often the one most difficult to achieve with trees. This phase, the stabilization phase, involves the development of shoot growth that is uniform, easily controlled by hormones in the medium, and readily subcultured. What physiologically occurs in the stabilization phase is not known, however the visible form of the shoot growth usually changes (leaf size, leaf shape, etc). What is apparent is that plant juvenility is involved (4). Stabilized cultures are much more readily obtained from juvenile stock plants than from large, mature trees. In addition, if mature tissue can be initially grown in culture, it will often show signs of 'rejuvenation' such as a markedly increased capacity for adventitious root formation. In any case, many trees cannot be micropropagated because they do not show uniform, continuous, and vigorous shoot growth after isolation and thus they will not fully

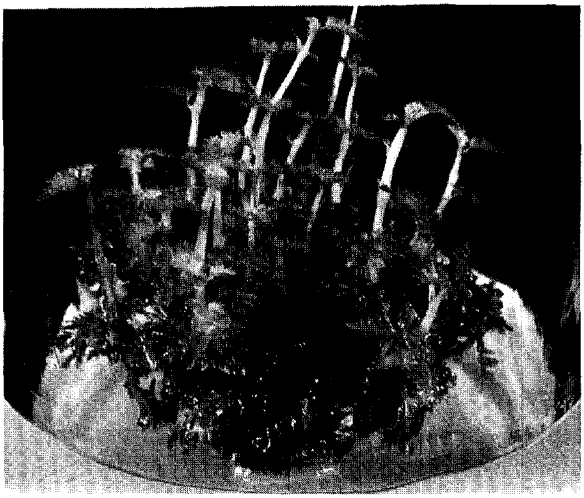

Figure 1. A shoot culture of a birch selection. This culture has entered its 'stabilized' phase and thus will continue to grow identical shoots after subculture for many years. This particular genotype has been in culture for more than a decade and the shoot cultures look identical to the first year. From cultures such as this, small shoots (microcuttings) are harvested, rooted, and acclimated for greenhouse or field planting (see Figure 2).

stabilize *in vitro.*

The third phase of micropropagation, the production phase, involves the maximal stimulation of shoots while at the same time maintaining high shoot quality. This phase is usually under the control of cytokinin hormones in the medium. In some cases, this phase may consist of two subphases, one for multiplication of shoots and the other for optimum growth of the shoots to maximize rootability in subsequent use of the shoots as microcuttings. If stabilized shoot growth can be obtained for a particular tree, then usually this phase is also obtainable.

Thus the major problem in developing a micropropagation system for trees is usually the stabilization of the shoot growth in culture. Since little is known about this phenomenon, we presently have limited alternatives to utilize to solve such micropropagation problems.

Table 1 gives some examples of trees that are presently being produced in commercial quantities by micropropagation. With those trees that have been micropropagated for some time and where we have been able to observe growth in the field (such as *Betula*), micropropagules appear to grow like seedlings (7) (Figure 2).

Just because a process employs a new high technology does not mean that some of the basic production and marketing rules that have prevailed for decades will no longer apply. For micropropagation in particular, the factors that make for a successful clonal production system are essentially the same as for the more classical propagation systems. The performance of the clone will for the most part be determined by its genetics; thus the 'junk in, junk out' rule applies.

Table 1. Some urban angiosperm trees that are being produced by commercial micropropagation laboratories.

I. Micropropagated plants used directly in production.
- -Amelanchier
- -Birch
- -Red and Norway maples
- -Tree lilac
- -Ornamental apple
- -Ornamental cherry
- -Ornamental pear
- -Poplar

II. Micropropagated plants used as propagation stock.
- -Elm
- -Maples

Micropropagation will not make an inferior genotype suddenly superior in field performance. Only the best of the selections, be they new or old introductions, should be placed into production as clones. In addition, quality control is as important with micropropagation as it is with budding. Proper monitoring and grading of the propagules is essential. In fact, these basic 'good practice' rules may be even more important for a micropropagation facility since a problem can be magnified so rapidly by the immense production (multiplication) capacity of such laboratories. Some problems have already arisen and include mislabeling, deformed root/collar systems, and genetic variation (mutation). The latter is particularly disturbing and has occurred with urban trees already (dwarfed maples and birches). Genetic changes in micropropagated clones are most often attributable to a lack of precise and adequate monitoring of the shoot multiplication process to avoid adventitious shoot formation as well as improper grading procedures. **Just like any other aspect of agriculture, the micropropagation industry must develop and maintain a high degree of professionalism and standards or it will not gain ready acceptance in the arboriculture—or any other—industry.**

Tree Genetic Engineering

When one looks at the trees that we use in our urban environments and considers their original source, it becomes obvious that trees are not a highly genetically-perfected crop such as most of our agronomic crops. Very few of our tree selections have been developed as a result of a highly sophisticated breeding program; most are the result of chance finds in the wild or in cultivated areas. The most common 'breeding' is selection from the open pollinated progency of promising parents. If one considers the immense obstacles that a tree breeder must face (long juvenile periods, large size, complex genetics, gross lack of basic inheritance information, a vast diversity of species in use), one is not at all surprised about this status. The question that is now being asked is whether plant biotechnology can give us tools that will enable tree breeders to more controllably create trees with the characters most desirable for the urban environment.

The answer to this question involves two subquestions: 1) Are the techniques available to genetically change trees using biotechnology? 2) Are the desired characters (genes) known and available for use? The first of these questions is rapidly being answered in the affirmative; the answer to the second question is much less optimistic.

The techniques to genetically modify trees involve an assortment of laboratory-based procedures, all of which have been borrowed from work on herbaceous species. The most refined technique for the transfer of genes is genetic transformation involving the insertion and expression of isolated genes. Genes can be inserted using either biological vectors such as *Agrobacterium* (crown gall disease) (5), protoplasts and direct gene uptake (1, 10), or microprojectile bombardment (micron size particles coated with the gene and 'shot' into the cell) (6). With trees, only the first process has been attempted on any reasonable scale; one result was the successful transfer to poplar of a bacterial gene coding for resistance to the herbicide glyphosate (2). At this writing, there is no reason to suspect that the other techniques will not be as successful with trees. In particular, the microprojectile bombardment procedure is promising because it diminishes the reliance on the complex *in vitro* tissue culture procedures such as protoplasts.

The aspect that makes the genetic transformation approach so exciting with trees is that we now have the capacity to add discrete and unique characters to our best specimens without changing all those inherent characters for which that specimen was selected. In other words, we have the capacity now to put a single character (or more) into *Betula platyphylla japonica* 'Whitespire Sr.' without changing this plants pyramidal character, its resistance to bronze birch borer, its heat tolerance, etc. For difficult-to-breed plants like trees, this provides an incalculable advantage in plant improvement programs.

A major limitation in the use of genetic transformation is that the technique is most efficiently used with isolated genes; that is, genes that have been identified as being important, have been removed from the source organism and multiplied, and have been properly tied to control elements so that they function in predictable ways in the recipient plant. This means that characters determined by many genes are not readily manipulated in this way. It also means that with trees, where our knowledge about the inheritance of various characters is minimal, we have little chance to work with 'tree genes'. For now, we must use the genes isolated from other better defined biological systems such as bacteria or herbaceous plants.

The above limitation may not be as severe as previous thought. Recent studies have shown that genetic transformation can be done with genetic elements as large as chromosomes. Such large segments of DNA can be transferred by microinjection (3) or through uptake by protoplasts

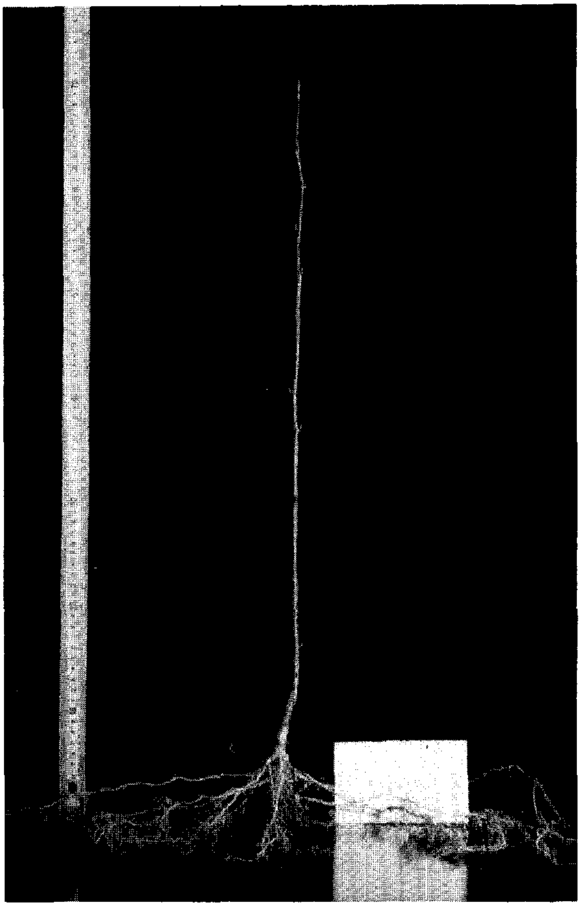

Figure 2. A one year-old birch plant grown from a micropropagule in a commercial nursery. This plant resembles birch grown from seedlings using identical cultural methods.

(Robert Griesbach, personal communication). Thus gene clusters located on the same chromosome may now be considered for biotechnological manipulations. As importantly, knowing the genes controlling a character or their exact location will not be as essential if individual chromosomes containing such genes can be manipulated as a unit in transformation.

Other approaches for the genetic modification of trees involve a diverse variety of techniques including haploid culture, somatic hybridization through protoplast fusion, and mutation induction through somaclonal variation. We now have evidence that these techniques may also be applicable for modifying tree germplasm, for example to increase disease resistance (9). Although unlike genetic transformation, the results of the manipulations using these techniques are not highly predictable and the resultant plants will still require extensive evaluation, selection, and breeding, such techniques can be highly useful when used in conjunction with a tree breeding program to speed the improvement process, especially where the genetic basis of the desired character is unknown.

All of the above genetic manipulation techniques require that a plant can be recovered from the cells or tissues that were modified. For some plant systems such as conifers, this is a major obstacle. However, for many of our angiosperm trees, when concentrated effort has been applied to solving the regeneration problem, success has been achieved. A good example is the progress in regenerating trees from protoplasts. Five years ago, only two genera of trees had been regenerated from protoplasts; in 1988, more than a dozen cases have been reported!

Some Problems Approachable with Biotechnology

As was discussed above, the problems that may be approached using biotechnological procedures depend in large part on the extent of our understanding of plant growth and interactions with the environment. For trees, because of a minimal amount of study devoted to these organisms, our understanding is currently very poor. This situation is not likely to change in the near future and thus for the most part, we will be limited to techniques and characteristics 'borrowed' from research on other more studied crops. One exception to this is the increased emphasis of forest research on biotechnology; much of the findings from these efforts will be translatable to urban trees, however many of the plant characters of importance to commercal foresters will not necessarily be of great value to arborists.

With micropropagation, we can see three important possible benefits to arborists:

1. Increased availability of superior selections. With those tree species that respond well to *in vitro* culture, improved selections can be cloned rapidly and thus brought to market in a minimum of time. For some trees, micropropagation may provide the only commercially viable method of cloning and thus production of the tree. As commerical micropropagation laboratories have become more widely established, they are actively seeking new cultivars to add to their products; this activity in itself has and will increasingly be stimulatory to new plant cultivar development and production, especially when one considers the additional stimulus of plant patenting. The final result to arborists is that there will be a wider range of superior cultivars from which to choose for urban planting.
2. More trees grown on their own roots. In those cases where micropropagation replaces budding as a cloning method, the trees will be on their own roots instead of seedling rootstocks of unknown origin and growth characteristics. This means that micropropagated trees should be more uniform in response and that suckering should be less common.

   When suckering does occur (as when the top of the tree is physically destroyed), suckers will be of the same genotype as the original planting. In addition, own-root trees can be evaluated and selected for their root characteristics as well as their shoot/trunk characters. Obviously, the problem of delayed graft incompatibility will no longer occur in micropropagated trees.
3. Preinoculation with beneficial microorganisms. Since micropropagation produces

very large numbers of small plants under laboratory conditions, the intentional inoculation of these propagules with microorganisms is facilitated. This may be particularly useful with mycorrhizal organisms or with bacteria that inhibit root-rot diseases. Of particular interest is the observation by many researchers working with woody perennials that material taken from the field appears to be commonly infected with bacteria living internally in the plant. This offers the opportunity of incorporating genes in naturally-systemic bacteria that will inhibit pests or disease organisms; such organisms can then be used to infect microcuttings of trees. Thus what one avoids is the necessity of having to engineer each tree species since at least theoretically, each tree selection can be infected with the same bacteria. Such plant/microorganism combinations are being actively researched with herbaceous crops.

In regards to genetic engineering of trees, four general benefits can be envisioned at this time:

1. Resistance to particular chemical agents. A number of characters are known to be determined by a few genes, some of which have already been isolated from organisms other than trees. Of particular note is herbicide resistance which may have minor significance to arborists. Whether genes for such stresses as salt tolerance will be identified and made available for general use is difficult to predict at this time.
2. Resistance to particular biological agents. Insect resistance genes have and are continuing to be identified. Resistance to lepidopteran pests is now readily achievable. Resistance to disease appears to be much more difficult to secure via biotechnology, although somaclonal variation may offer an intriguing approach. With all of the resistance to biological agents, the target pest must be chosen very carefully so that the chances of promoting the development of pest tolerance to the source of resistance is minimized. This is a particularly important problem with long-lived perennials such as trees.
3. Sexual sterility. One intriguing prospect is a possibility of being able to make a superior selection sterile. This not only would mean less stress on the plant because of the elimination of a drain on the tree's reserves, but some species (eg. oaks) would have a wide use because of the elimination of an aesthetic problem (eg. messy fruits) or a hazard (eg. acorns on sidewalks. At present, genes that would accomplish this are not readily apparent, however it is not difficult to envision that such genes exist and could be isolated. In reality, sexual sterility may be a prerequisite of most genetically-engineered trees because of regulatory restrictions on the inadvertent distribution of foreign genes present in engineered plants.
4. Change in plant form. Characteristics like dwarfness, pyramidal shape, and compactness are not difficult to envision as approachable by insertion of genes controlling hormone production and sensitivity or by somaclonal variation. Compact forms of some ornamental shrubs (rhododendrons) have already been isolated using somaclonal techniques. How much one can manipulate the hormonal status of a plant without altering critical growth processes has yet to be fully studied, especially for perennial species that must be intricately attuned to the seasonal environmental cues.

Conclusions

Biotechnology may indeed be a part of the 'magic wand' that arborists need to solve some perplexing and continuing problems with urban tree management. Two major obstacles to the use of biotechnology for urban trees is our lack of basic knowledge about urban tree growth and ecology and the paucity of biotechnological research activity on urban trees. Thus one is presented with a frustrating dilemma: we have a powerful tool, but when will we be able to use it?

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Department of Horticulture
University of Wisconsin
Madison, Wisconsin 53706

Abstracts

RAUPP, M.J. and C.F. CORNELL. 1988. **Pest prevention.** Am. Nurseryman 167(3): 59-62, 65-67.

Monitoring is the process of regularly inspecting plants for pests and adverse environment conditions that affect vigor, form and quality. For several decades, monitoring has formed the backbone of management programs for traditional agricultural crops. Monitoring can do more than reduce pesticide use. It can also supply the detailed information necessary to time control actions for maximum effectiveness. Environmental factors, such as temperature and rainfall, directly affect the rate pests develop and grow. And most controls are most effective on particular pest stages. Many monitoring techniques and approaches are currently available. They fall into three general categories: visual inspections, traps and predictive models based on environmental monitoring.

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Services are much harder to describe than products. Standards are not as clear cut, and the thing a consumer is trying to measure doesn't sit still. Services are intangible. They are also variable, in several ways. The service itself varies—people simply perform better on certain days and certain types of projects and with certain customers. The perception also varies. If you were to perform exactly the same service for five different customers, you would probably get five different ratings for your service. Customers see, hear and react to things in their own unique ways. Service is intangible, variable and hard to differentiate. Therefore, you can't market a service the same way you would market a product. That is why it is crucial to decide which it is you are offering. Most service organizations find that well over half, often 75 percent, of their volume comes from repeat business from existing clients. Many find that all of their business comes from existing clients and their referrals. These people think that they aren't marketing. In a systematic sense, they're not. However, these observations lead to but one conclusion: The most important marketing tactic for the service provider is to constantly give superior service.