1974

# Toward The Future Forest: Applying Physiology And Genetics To The Domestication Of Trees

J.H Rediske

F. Thomas Ledig

Peter S. Carlson

Thomas B. Rice

Richard P. Pharis

## Recommended Citation

YALE UNIVERSITY: SCHOOL OF FORESTRY AND
ENVIRONMENTAL STUDIES

BULLETIN No. 85

TOWARD THE FUTURE FOREST:
APPLYING PHYSIOLOGY AND GENETICS TO THE
DOMESTICATION OF TREES

BY

J. H. REDISKE
F. THOMAS LEDIG
PETER S. CARLSON AND THOMAS B. RICE
RICHARD P. PHARIS

Edited by F. THOMAS LEDIG

# A Note to Readers
# 2012

This volume is part of a Bulletin Series inaugurated by the Yale School of Forestry & Environmental Studies in 1912. The Series contains important original scholarly and applied work by the School's faculty, graduate students, alumni, and distinguished collaborators, and covers a broad range of topics.

Bulletins 1-97 were published as bound print-only documents between 1912 and 1994. Starting with Bulletin 98 in 1995, the School began publishing volumes digitally and expanded them into a Publication Series that includes working papers, books, and reports as well as Bulletins.

To celebrate the centennial of publishing at the school, the long out-of-print Bulletins 1-97 were scanned to make them available as pdfs to a broader audience. *A caution: the scanning process is not perfect, especially for print documents as old as some of these, so the readers' indulgence is requested for some of the anomalies that remain despite our best efforts to clean them up.*

Everything published from 1912-present is available on the School's website (**http://environment.yale.edu/publications**) for free download. Nothing in the Series requires copyright permission for reproduction when intended for personal or classroom use.

Bound copies of everything published in the Series from 1912 to the present are also available in the Yale University libraries and archives and can best be accessed by contacting the School of Forestry & Environmental Studies librarian.

# TABLE OF CONTENTS

# THE CHAMPION INTERNATION CORPORATION LECTURE SERIES

## PREFACE

A century and more ago, America was cutting the first forest, the wild or so-called virgin forests. In time, the forest regenerated naturally and the second growth was cut in turn. Today's production forest, or as it is called, the third forest, is growing from trees planted by man on physically manipulated sites-sites on which species composition, spacing, fertility level, and competition are controlled. In tomorrow's forest, the future forest, not only will the environment be manipulated, but the genetic makeup of the trees themselves, or their genotype, will have been created and controlled by man. This, the fourth forest, a truly domesticated forest, will be the result of man's attempt to rework the genetic material and recombine it in packages never before seen or possible under natural conditions, existing only by virtue of man's prowess as a breeder.

In agriculture some of the earlier developments in crop domestication are lost to memory in pre-history. Tree breeding as such is a much newer endeavor and relative to the development of agricultural crops, corresponds to that of early neolithic man. Before the turn of the century it was not necessary for man to actually raise and culture trees for building materials and fibers.

In this country, "forestry" was a purely exploitative operation and its practitioners needed only a knowledge of logging. However, vast areas of timber are no longer available for cutting. Today, the heavy demands for recreational and esthetic benefits of the forest, combined with a loss of forest land to urban uses, necessitate production of timber and pulp on a restricted land base.

Therefore; productivity must be improved. In this endeavor, forest genetics and tree breeding have a major role through the multiplication of the better timber types now available and particularly through the creation of new types that are superior to their wild ancestors in those characteristics valued by society. The future forest will be one in which domesticated trees predominate. The development of such trees is what this lecture series is about.

Dr. John H. Rediske, in charge of forest tree improvement research for the Weyerhaeuser Company, is in the forefront of programs designed to capitalize on the science of genetics and physiology. His concepts of the direction tree improvement must take led to this series of four Champion International Lectures. In his lecture, Dr. Rediske presented the objectives of the genetic approach to tree improvement and recounted some of the successes and mistakes that have marked similar endeavors in crop breeding. He defined the areas of biological research that will contribute most to future development in tree improvement. These areas were considered in the next three lectures by F. Thomas Ledig, of the Yale School of Forestry and Environmental Studies, on "Photosynthetic Capacity: Developing Criteria for the Early Selection of Rapidly Growing Trees"; by Peter S. Carlson and Thomas B. Rice of the Brookhaven National Laboratory in Upton, Long Island, on "The Potential of *In Vitro* Techniques for Forest Genetics"; and by Richard P. Pharis of the University of Calgary, Alberta, Canada, on "Precocious Flowering in Conifers: The Role of Plant Hormones". These lectures were made possible by a grant received from the Champion International Corporation in 1967.

<div style="text-align:right">

François Mergen
Dean and Pinchot Professor of Forestry
Yale School of Forestry and Environmental Studies
December 4, 1973

</div>

# THE OBJECTIVES AND POTENTIAL FOR TREE IMPROVEMENT

J. H. Rediske
Forestry Research Center,
Weyerhaeuser Co.,
Centralia, Washington 98531

*Introduction*

**In** the perhaps 2 million years that man has been on this planet, certainly one of the most important contributions he has made to his continued existence has been the domestication of plants. The process of domestication was initiated in early Neolithic times with certain grasses and has continued with one species or another, as the opportunities became obvious to man, until the present.

This series of lectures is devoted to the domestication of trees, particularly those of the Pinaceae. The process of domestication in trees will follow the familiar pattern discussed in detail by Libby (1969, 1973). This process includes the growth of individuals in plantations of a monoculture or near monoculture so that they may be efficiently maintained. Maintenance is usually intensive so that individual plants may be stimulated for maximum productivity.

A process of evolution occurs simultaneously. Initially, plants are screened by natural selection for survival and growth in a plantation environment. Eventually man takes over the selection process to consciously favor those individuals for seed which closely approach his ideals. These ideals usually relate to productivity, but additional factors such as disease resistance and form have been considered.

Even though the domestication process is now well understood following a great many repetitions, some misunderstanding of the process as applied to forest trees seems prevalent. Perhaps the misunderstanding stems from the long use of our forests in the wild state at the level of a hunter-gatherer society. There was plenty of wild wood available to satisfy man's requirements, so there was little incentive to intensively manage plantation forests. Or perhaps modern man is aware of the mistakes that have been made with the

domestication of other crops and is apprehensive that similar mistakes will be made with forest trees. In either event, a few words in justification of the domestication process for forest trees might be in order.

Of singular importance is the fact that the once endless supply of wild wood on forest lands has now become visibly finite. Furthermore, the continuing pressure of expanding urban populations has brought about a number of often competing uses for what forested land remains. A system of national priorities appears necessary to allocate our forest lands for those necessary functions of watershed, recreation, natural areas, and a multitude of other uses, surely including the production of wood.

Wood as a raw material for the production of building products, packaging materials, paper and fiber products has attained a position of significance and universalness rivaled by few other products. Wood is a renewable resource. The only limit on its availability, is the amount of land available for its continuous production. Wood as a product is not a drain on our finite supply of raw materials. The required raw materials for tree growth are carbon dioxide and water which are continuously renewable in the biological cycle. Wood as a product is not significantly dependent on our increasingly scarce energy supply. In fact, it is conceivable that a wood processing industry could be developed which was largely independent of external energy sources from tree to finished product, either lumber or paper. This is possible because the energy-rich wastes from the total process of growing and manufacturing wood, such as hog fuels, could be used for the internal production of steam or electricity to carry out the manufacturing process. As the cost of traditional fossil fuels increases, greater efficiencies will develop in the utilization of forest residuals now left in the field because they are not economic to remove. Wood, it develops, while being of use to man from antiquity, is also a modern, renewable product fitting in well with the recognized need to conserve both energy and nonrenewable resources that the products which compete with wood require in large amounts.


*Domestication of Forest Trees*

Once a priority has been established for the allocation of forest land for various national needs, the productivity of those lands set

aside for wood production as a primary use must be closely examined. It is apparent that average wild stands are growing at only half the rate possible through application of domesticated management practices utilized by plant husbandmen for centuries (Staebler 1972).

These practices are generally well known and with few exceptions rather universal regardless of the domesticated species. A primary step in the process of domestication is the establishment of plantations, a prerequisite for the efficient application of subsequent domestication processes. Spacing of individuals within the plantation is a necessary step for optimum productivity. Competition from weed plants, those not belonging in the plantation, must be controlled. Shaping of the plant by pruning or by spacing in the plantation often improves productivity or the value of the harvested product. Certainly, the application of fertilizers and the supplemental addition of soil moisture are proven domestication procedures. Intermediate harvests before the final harvest will often increase the total productivity of a plantation. All of these practices are utilized by man to husband crops for the efficient production of plant products for his needs. There is no reason to suspect that forest trees will be different in their general domestication requirements or that the rewards of domestication will be any less. Information available to date supports this contention (Staebler 1972).

*Tree Improvement*

There is one very important aspect of domestication which has not been discussed. That aspect is the application of the science of genetics or the art of crop breeding to improve the productivity of the domesticated plant. With most other crops, domestication by cultural practices was carried out long before man understood the Mendelian principles. With forest trees, man has the opportunity of developing cultural practices and breeding improved plants concurrently. The simultaneous exploitation of both techniques should provide a more efficient domestication process, because plants can be consciously bred to perform well under specific cultural practices rather than placing reliance upon the slower evolutionary shaping of the species.

There are some problems in applying the technology of selection and breeding to forest trees, however. The problems are generally related to the large size of individuals and long generation times. The long generation not only affects the length of time to reproductive maturity but increases the time necessary to evaluate each succeeding generation. Most of the characteristics desired as objectives of a breeding program are those associated with the mature plant, such as wood volume. Methods of circumventing these disadvantages must be a key element of the breeding strategy in any domestication program for tree species.

There are advantages as well as disadvantages that accrue to present domestication programs in forest trees. Because domestication has been practiced for many years with other species, many common mistakes have been recognized and can be avoided in tree breeding. Attempts to increase uniformity in corn by decreasing the genetic base through inbreeding and the production of single crosses planted exclusively over entire regions has precipitated many problems with disease resistance (Hooker 1972). Tree breeders should not make this mistake (see Harlan 1972).

The loss of the original wild population of many domesticated plants has been a serious handicap to crop breeders. The variability in the original wild population would have been of great help in combating susceptibility to insect pests, diseases, and other environmental factors (Libby 1973). Thankfully, the maintenance of forest gene resources is a topic of great current interest. A solution to the potential problem of loss of variability would be the reservation of natural stands of forest species in an area large enough to maintain vigorous populations. This approach fits in well with at least one other priority use of forest land, the establishment of natural areas.

With those lands allocated to wood production, and perhaps most private forest lands fall in this category, the possibilities of contributing to the national wealth by the domestication of certain species, particularly in the Pinaceae, are significant. The opportunities are clearly defined. The experience gained through centuries of plant husbandry can be wisely combined with today's technology to domesticate some of our forest species, resulting in a significant benefit to mankind. **In** the remainder of this paper the concepts and processes involved in the domestication of the Pinaceae are discussed.

## The Empirical Basis for Tree Improvement

In order to understand the motivation for a breeding program for forest trees I will begin not in the forest, but in the cornfield. Iowa corn country is where some of agriculture's most spectacular successes have been achieved. Yields have increased over the past 30 years, not by percentages, but by multiples. Fertilizers, chemicals, irrigation, and better management have all been responsible. But especially significant has been improvement due to genetic change.

**CORN YIELDS/U.S. Average**

Bu./Ac.

*Intensive Farming*
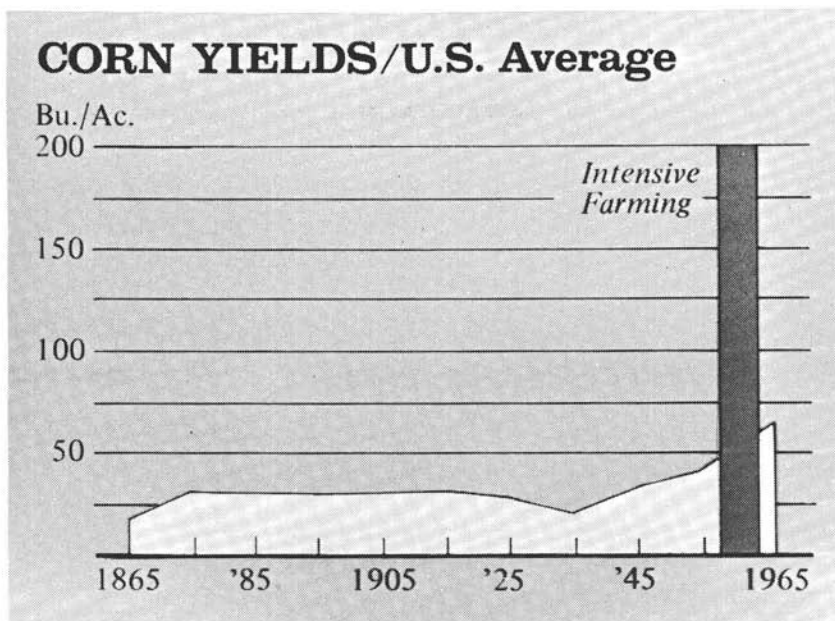
Figure 1. Corn yields in the United States from 1865 to 1965.

Figure 1 reveals how significant the combined effects of these gains have actually been. The chart shows the average national corn production over the past one hundred years. From 1865 right up to World War II there was not much variation. The average ran between 10 and 30 bushels per acre. But, since the forties, that average has more than doubled to 65 bushels per acre. Even more spectacular is the bar showing not the average figure, but the production that truly intensive farming can produce—two hundred bushels per acre

–**and** there is serious suggestion this may be increased to 300 bushels *(V.S.D.A.* 1965, Kohnke and Miles 1951).

The origin and the development of corn has occurred in our own hemisphere. The original wild maize plants have long since disappeared and we can only guess at the origin of the species. But from the early beginnings, maize was maintained and developed by selection by the Indians of Central America and northern South America. From there, it spread over most of the potential corn-growing region of the hemisphere. A good record is left in prehistoric Indian dwelling sites of stages in the development of maize by selection (Mangelsdorf 1947, Mangelsdorf *et al. 1964).*

From Bat Cave in New Mexico, six successive time layers from approximately 2500 B.G to 500-1000 A.D. have been excavated. These layers show a progressive increase in size of not only the kernel but the cob as well, so that in an ca. 3000-year interval, the size of the seed as well as the diameter and length of the cob have increased approximately three times. The increase in yield was certainly brought about by selection processes, including a genetic response to the plantation environment. One cannot discount, however, the possibility that some increase in yield was due to a cultural environment that improved with time. Nevertheless, the conscious contribution of the modern plant breeder to genetic improvement of corn has been great.

**If** one assumes that ear and kernel size are directly related to yields per acre and that the productivity at 500 A.D. was little different from the average at 1865, then during the last 4500 years, corn productivity has increased about 8 times on the average. **If** we compare this to production possible under intensive management (at least another factor of 2) the yield has increased about 16 times from the primitive, near-wild corn plant to the modern plant under intensive management.

Outstanding gains have been made in many other crops, among which wheat is a good example. Over 100-years ago the national average production was 10 bushels per acre. Since 1940 the average has increased to 22 bushels per acre, while intensive farming can boost yield to as much as 90 bushels. (U.S.D.A. 1965, Kellogg 1964). Here again, the contribution of genetics has been most significant.

Similar gains in tree growth are unknown and efforts toward achieving them are in their infancy. Trees are still wild plants, molded by evolution only to survive and reproduce. Their improvement,

8

in a genetic sense, is less advanced now than was corn 45 centuries ago. In view of the many-fold increases in agricultural yields, it seems reasonable to assume that improvement is feasible in forest trees. But how will this improvement be brought about?

Because the process of genetic improvement takes many years, the importance of improving the right characteristics is vital. There are several examples in agriculture of choices that turned out to be the wrong ones. Some years ago a premium price was paid for milk with a high butter fat content, and aggressive breeding developed dairy cattle able to produce high-fat milk. Jersey and Guernsey breeds were in favor. Then the public turned to weight-watching, the correlation between cholesterol and heart problems was revealed, and suddenly the market opportunity was not in butter and cream, but in low-fat products. Farmers switched to Holsteins for their quantity milk production, rather than for any particular quality of the milk. The forecast of future markets had been wrong, and so the once-favored breeds of cattle were wrong, too. Enormous changes have also occurred in the forest products industry. For example, the one-time orientation to lumber production has partially shifted to fiber. So, what should a program of tree improvement seek to attain?

Virtually any characteristic with the basic capability of being passed from parent to offspring can be emphasized. We can begin by looking at some of the criteria identified by others. Straightness was one of the first selection criteria in forest trees because of its effect on lumber production. But, as we move to a more complete utilization economy, straightness has less significance. Another case is selection for an increase in gum yields in Florida. The importance cf the program rests on the security of the market for tree-derived turpentine, now dependent on government subsidy. Wood properties have also come in for considerable attention in tree improvement programs. Wood density and fibre length have been used as selection criteria, particularly in loblolly pine *(Pinus taeda* L.) programs. Southern seed orchards are now in existence that have been created to enhance each of these characteristics.

In my view, the principal requirement for the long term will always be wood quantity. So the objective should be to breed trees with a high capacity for growth. This does not mean they will look different from today's trees, but they will lay down wood faster, and so their growth rings will be relatively wide. Intensive management practices are already increasing ring width. These same practices

applied to trees inherently capable of rapid growth will make them wider still. Forest industries have a responsibility to develop the inherent capability for rapid growth on industrial forest lands.

## *The Scientific Basis for Tree Improvement*

The tree improvement process draws on several fields of biological discipline. The study of genetics is fundamental. Basic to all the other processes in tree improvement must be an understanding of the science and art of manipulating inheritance. Not only is the ability to see that the right characteristics are transmitted from generation to generation required, but also the ability to somehow enhance the desirable characteristics to the limit of variability. Physiology is also important to the tree improvement process because most of the functions to be controlled are physiological in nature. Reproduction, in particular, is a process poorly understood but vital in its impact on tree breeding. Ecology is a necessary discipline since the strains of trees that we develop are literally new to nature yet they must be able to perform in a wide range of environments. The valid assessment of the performance of these strains in the field is an important part of the tree improvement process. Fourth and last, even though we are selecting for growth performance, we have the opportunity to examine wood properties in the trees we produce. We must know how wood characteristics change, as changes occur in the trees' growth habit. Most of the research necessary to a tree improvement program will draw on the four disciplines-genetics, physiology, ecology, and wood science.

## *The Process of Tree Improvement*

The ultimate practical objective in tree improvement is to produce seeds, from which the high-producing forest will be grown. Seed production can be accomplished by the establishment of seed orchards. The steps involved in tree improvement, from the selection of parents with high wood producing characteristics until they are producing seed in a seed orchard, will be examined below.

**In** developing new horticultural varieties, hybridization has been highly successful. Usually, results have been achieved by controlled

mating between species, as for example, between different species of rhododendron. The greatest potential for improving forest trees, however, lies in hybridization within species. Intraspecific hybridization involves the mating of individual trees from widely separated parts of the species' geographic range. Such crossing experiments are already underway with several species.

However, the most promising approach is through the process of selection, not hybridization. The process will be outlined as applied to Douglas-fir *(Pseudotsuga menziesii* [Mirb.] Franco). The selection process takes advantage of the natural variability of certain tree characteristics. One of these is growth rate. Even when grown under as nearly identical conditions as possible, some individuals will perform better than others. Convincing evidence of this variation comes from a Douglas-fir heritability study, initiated with remarkable foresight by Thornton Munger in 1912 (Munger and Morris 1936). Fifty years later it was apparent that in timber volume, the best families had out-produced the poorest by ratios of two and even three to one (SHen 1966).
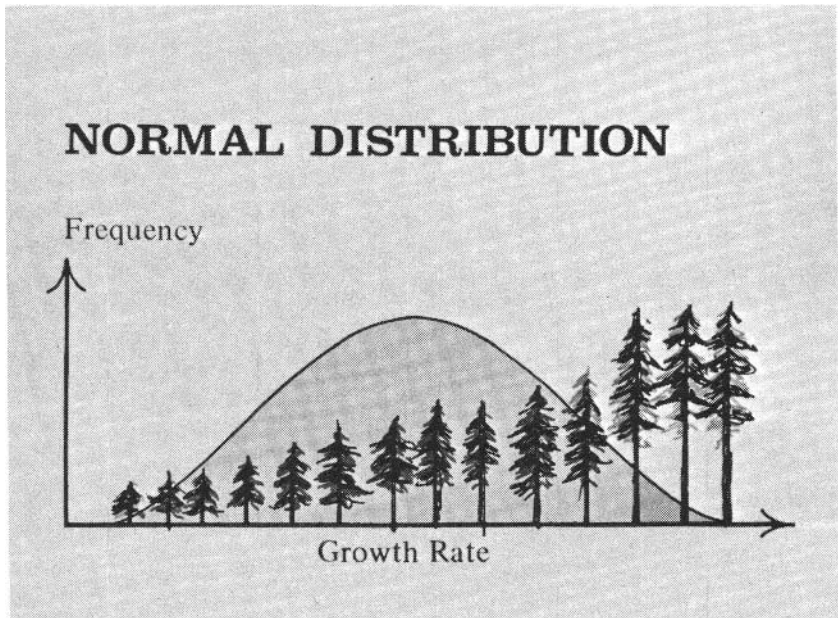


Figure 2. Frequency distribution for **growth** rate in a hypothetical population of trees.

If rates of growth are plotted against the frequency of each rate's occurrence, the result in a tree population is a normal distribution curve (Figure 2). Most individuals lie near the mean. Trees with slow rates of growth are at the left, and those with rapid rates of growth at the right. The trees with rapid rates of growth, "plus-trees", are the trees that we must identify to begin our tree improvement process. Initially, high-quality stands of wild trees are measured, and individuals are identified that utilize the space available to them most efficiently. The process usually involves a statistical sampling procedure relating several environmental factors to the growth of each tree. The plus-trees, as they are called, are not necessarily the biggest, nor the best-looking trees in the stand, but are the top-performing wood producers when environmental factors are eliminated. The plus-trees must be stratified both geographically and elevationally and kept separate in subsequent operations so that their offspring may be planted back into similar environmental conditions.

Once the trees have been identified in the forest, branches are removed from their crowns, and subsequently 3-4 inch twigs, called scions, are prepared for grafting. Meanwhile, seedlings have been raised in pots at the seed orchard site to serve as grafting stock. Now the scion material collected from wild plus-trees is grafted onto the top of each stock seedling, and bound in place. When union of the tissues is accomplished, the trees are planted out in orchards, stratified according to geographic and elevational origin. Seeds are produced as a result of controlled mating. Pollen collected from the male strobili on one tree is injected by syringe into bags covering the female strobili on another tree. The bags are necessary to exclude wild pollen carried by the wind from unselected parents. Every cross is planned, and fully documented. By the end of the summer, seed have matured, at least in Douglas-fir, and can be extracted from the cones. In the initial years of the orchard, the earliest seed produced is used to evaluate the breeding value of the parents by progeny testing. The seed from the scion of trees selected in natural stands should be capable of producing trees with an expected gain in growth, over average wild trees, of 10-20 percent as reported by the North Carolina State University Cooperative Tree Improvement Program (1973). Progeny testing is conducted to escalate the gain. Only those plus-trees actually producing superior progeny will be selected to remain in the orchard, the rest will be rogued.

To understand this, refer to the bell-shaped curve in Figure 3.
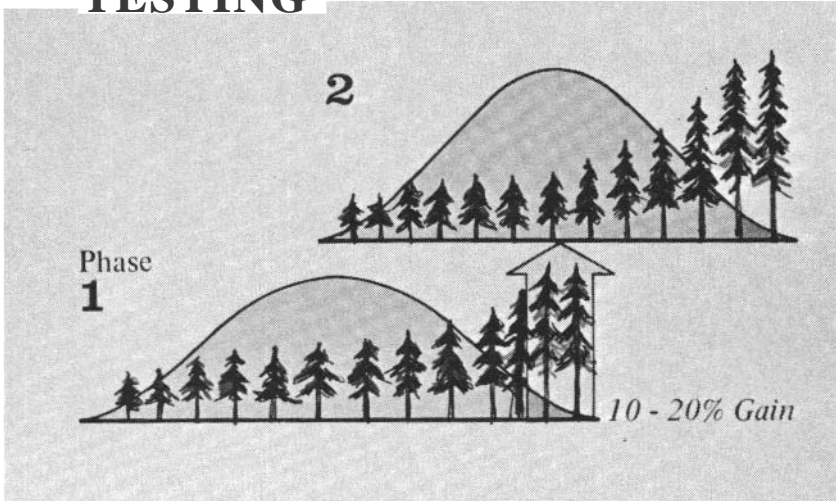
# PROGENY TESTING



Figure 3. Shift in the frequency distribution for growth rate in the progeny (phase 2) of high-yielding trees from the original wild population (phase 1).

Originally, we selected the best trees we could find in the wild from the right of the lower curve, grafted them into a seed orchard, and mated the high-yielding males with the high-yielding females. Their offspring, or progeny, are represented in the upper bell-shaped curve. Again, the progeny form a normal distribution. Some individuals will perform less well than either of their parents, and these are at the left-hand end of the curve. But, those at the right-hand end are better than either of the parents. The time-wait before the seedlings grow enough to make evaluation possible is considerable, at least five and possibly fifteen years. The longer the wait, the more certain the conclusion on which parent trees produce the best progeny.

A second breeding generation is selected from within the progeny test. Cuttings are taken from the selections and grafted into a new Phase 2 seed orchard. In time these trees too will flower, and controlled mating of male and female will again produce a new generation of seeds. There will be an additional gain in growth rate of the second generation, over average wild.

This sequence is followed through successive breeding phases. Each generation will have a higher mean growth rate. What the gain will be after a specific number of breeding phases is not actually known, but the North Carolina State University Tree Improvement Cooperative has predicted a second-phase gain of 35-45 percent. Perhaps a doubling in wood production may be attainable from an advanced breeding orchard. The bell-shaped curves may change somewhat in shape with the progression of the program, and become steeper, with a narrower base, as variation in growth rate is reduced. But selecton for growth will not *ipsofacto* change the breadth of the base for other characteristics such as response to the environment, or response to insect or disease attack.

### Accelerating the Tree Improvement Process

The principal drawback with a program of the sort described is the time involved. Conventional tree improvement practices would require more than 50 years before a fourth phase orchard came on stream. The time-wait has been a fundamental obstacle to the serious pursuit of tree improvement by industry. How can we realize the higher gains available by four breeding phases, for example, at a much earlier time? There are three primary areas where research can be directed. New technology developed in early progeny ranking, vegetative propagation, and flower initiation would be most helpful. I shall discuss each of these in turn.

From the description of the steps in the improvement process it should be obvious that progeny testing is very important, yet very time-consuming. When the wild plus-trees were selected in Figure 1 from the right-hand end of the lower curve, they were mated and the offspring grown for 5, 10, or 15 years before the best performers among parental or progeny generations could be identified. A tremendous advantage could be gained if this time consuming procedure were shortened from 15 years to perhaps 15 months. To accomplish this aim, much work has been done on the relationship of the photosynthetic process to growth potential. Such a relationship exists because growth in a tree is a direct result of that tree's ability to take carbon dioxide from the air, combine it with water with the aid of energy from the sun, and produce tissue. This is the process of photosynthesis. **It** follows that if growth is related to net photosyn-

thesis and net photosynthesis is measured by carbon dioxide fixation, then a measurement of $CO_2$-uptake should provide an index to growth performance (Ledig 1969). The use of some measure of the photosynthetic ability of a progeny offers a great possibility for evaluating its potential at a very early age. The exploration of this possibility is the subject of a subsequent paper in this volume.

A second possible approach to early progeny ranking is the measurement of height or some other growth characteristic of very young seedlings grown in a growth chamber or greenhouse environment. Some correlations have been demonstrated between growth performance of very young seedlings and their later ranking under field conditions, but much work remains to be done to develop a fully usable technique. A third approach is to grow numbers of seedlings from each progeny in a nursery environment. Progeny or family selections can be made on the basis of growth comparisons. Finnish foresters in particular have utilized this method. Unfortunately, all current methods of early testing have lacked sufficient precision to justify replacing the standard field test. The longer one waits and the closer the approach to harvest time, the more accurately can progenies with the greatest growth potential be recognized. Though early progeny ranking has not yet proven itself, its potential for savings in time are tremendous and perhaps intensified research will yet develop a suitable technology.

A second time-saving technique in the tree improvement process is possible through improved technology in vegetative propagation. If we are able to identify the best performing trees by early progeny ranking, we must still multiply these trees rapidly and establish the next phase orchard. Current practice is to graft scions of the identified superior trees onto rootstock as previously described. Often, however, a curious incompatibility develops between rootstock and scion which at least weakens the union and often results in death of the tree.

To compensate for this, a technique of double grafting is employed, using a terminal and a lateral graft of the same scion material. After a suitable period of growth, usually two years, the terminal graft is sacrificed and examined by histochemical techniques. Wound tissue, evidenced as darkly stained wood near the center of the stem, indicates incompatibility. If the graft was compatible the lateral graft would be allowed to grow and produce the orchard tree. If not compatible, suitable graft-scion combinations must be found. An al-

ternative method of vegetative propagation is by means of rooted cuttings. But many conifers, particularly Douglas-fir, have rooted with difficulty or erratically. After much research, it was possible to root most conifers, especially juvenile material.

Perhaps the answer to vegetative propagation lies with tissue culture techniques. So far quaking aspen *(Populus tremuloides* Michx.) is the only forest tree successfully propagated by tissue culture techniques (Winton 1968). Stem segments of aspen are placed on a nutrient medium which stimulates the growth of callus tissue from each end. Bits of this callus tissue are placed on another medium which causes it to grow and differentiate into root, stem and leaf tissue, a plantlet. The plantlet can be transferred to soil and eventually develops into a normal tree.

Tissue culture methods can conceivably have an impact on tree improvement far beyond their use as a means of vegetative propagation. Technology is developing to the point where the fusion of haploid somatic cells of tissue culture origin may produce the same result usually achievable only by breeding. Truly, the technology of tissue culture has the potential for spectacular impact on tree improvement. This fascinating work is discussed in the paper by Carlson and Rice in this volume.

A third area of concern in saving time is flower initiation. If we can short-cut our approach to identifying the most efficient, most productive seedlings, and we can multiply them successfully for subsequent new, advanced phase orchards, we must still wait for these seedlings to produce both male and female strobili. The problem is that Douglas-fir and many other conifers seldom reproduce before 10 to 20 years.

Most trees seem to have both a juvenile and a mature stage. The juvenile state is not reproductive but vegetative in its growth patterns. Many physiological differences exist between juvenile and adult tissues. For example, stem tissue roots very well in the juvenile stage. Tissue fully mature roots with difficulty but develops reproductive tissue readily. Little is understood about the factors influencing the change from the juvenile to the mature state or the reverse in most conifers. Somehow the juvenile nonreproductive period of conifers must be compressed in time so that orchards will be productive at an earlier age.

With some species, such as Arizona cypress *(Cupressus arizonica* Greene), reproductive development has been defined as a hormonal

problem. Selective application of specific gibberellins has been successful in inducing the reproductive stage in young cypress. Even more spectacular is a female cone produced on the tip of a four centimeter high western redcedar *(Thuja plicata* Donn.; Owens and Pharis 1971)*. If through research we can understand the hormonal differences between juvenile and mature tissue, we could conceivably cause them to produce seed at will and considerably shorten the time required to reach advanced phase orchards. This intriguing subject likewise is discussed in a paper by Pharis in this volume.

The three areas of research that I have just described, early progeny ranking, vegetative propagation, and Hower initiation are all designed to speed up the conventional routine of tree improvement. Success in these areas would make it possible to attain the advanced phase orchards at a much earlier time with a consequent increase in wood productivity.

But while we are doing this, we must also culture and manage each orchard, as it is established, to produce seed in maximum quantity. Fortunately, it seems highly probable that seed production can be boosted in the operational orchards over production expected in wild stands. Additional research is required on frost control, limitation of insect damage, fertilizer and irrigation regimes, and other seed production research activities.

If we accomplish the research objectives described here, we will have significantly shortened the tree improvement process. Tree improvement is a key activity in the domestication of the important forest trees. The challenge is clear. The research to date is promising. In the long view the domestication of the Pineaceae may not be as important to man as the domesticated Gramineae, but the potential rewards to man are great nevertheless. The Pineaceae are wonderfully adapted as fiber producers with great potential for increased productivity. Shelter and clothing, next to food, represent mans greatest physical needs.

## LITERATURE CITED

Harlan, J. R. 1972. Genetics of disaster. J. Envir. Qual. 1: 212-215.

Hooker, A. L. 1972. Southern leaf blight of corn-Present status and future prospects. J. Envir. Qual. 1: 244-249.

Kellogg, C. E., 1964. Potentials for Food Production, p. 57-69. *In* Farmer's World. The Yearbook of Agriculture, 1964. Washington D.C.

Kohnke, H. and S. R. Miles. 1951. Rates and patterns of seeding corn on high fertility land. Agr. J. 43: 488-493.

Ledig, F. T. 1969. A growth model for tree seedlings based on the rate of photosynthesis and the distribution of photosynthate. Photosynthetica 3: 263-275.

Libby, W. J. 1973. Domestication strategies for forest trees. Can. J. For. Res. 3: 265-276.

Libby, W. J., R. F. Stettler, and F. W. Seitz. 1969. Forest genetics and forest-tree breeding. Ann. Rev. Genet. 3: 469-494.

Mangelsdorf, P. C. 1947. The origin and evolution of maize. Advan. Genet. 1: 161-207.

Mangelsdorf, P. C., R. S. MacNeish, and W. C. Galinat. 1964. Domestication of corn. Science 143: 538-545.

Munger, T. T. and W. G. Morris. 1936. Growth of Douglas-fir trees of known seed source. U.S.D.A. Tech. Bull. No. 537. Washington D.C. 40 p.

North Carolina State University Cooperative Tree Improvement and Hardwood Research Programs. 1973. Seventeenth Annual Report. North Carolina State Univ., Raleigh, N.C.

Owens, J. N. and R. P. Pharis. 1971. Initiation and development of western red cedar cones in response to gibberellin induction and under natural conditions. Can. J. Bot. 49: 1165-1175.

Silen, R. R. 1966. A simple, progressive, tree improvement program for Douglas-fir. U.S.D.A. For. Service, Pac. N. W. For. and Range Exp. Sta. Res. Note 45. 13 p.

Staebler, G. R. 1972. Genetics as a forest management tool; AlES National Meeting, Washington D.C., December 28, 1972.

U.S. Dept. of Agriculture. 1965, 1950, and 1938. Agricultural Statistics. Washington D.C.

Winton, L. 1968. Plantlets from aspen tissue cultures. Science 160: 1234-1235.

# PHOTOSYNTHETIC CAPACITY: DEVELOPING A CRITERION FOR THE EARLY SELECTION OF RAPIDLY GROWING TREES

F. Thomas Ledig

School of Forestry and Environmental Studies,
Yale University,
New Haven, Connecticut 06511

For the purpose of this review, tree improvement means the production of more rapidly growing trees than those now available. Among present wild populations of forest trees, some individuals produce progeny inherently more rapidly growing than those of others, even when provided with similar environments. For several years, research in our laboratory has been directed to the questions: Why do the progeny of one tree grow more rapidly than those of another, and can superior growth be predicted at an early age? This paper will indicate why such questions are of practical importance, review the hypothetical framework, or model, constructed to explain growth and the investigations by which the model was tested, and discuss the application of the results. The review deals entirely with work conducted in the forest genetics program at Yale University and is not intended to be exhaustive.

## The Importance of Early Evaluation

The pattern of most tree improvement programs is to make initial selections from the wild, intercross the selections in one of several possible mating designs, grow progeny of the crosses until they reach harvest age, and make final selections among the parents based on their progenies' performance. Those parents whose progeny produce the highest yield at harvest are retained for breeding and for the production of improved planting stock. In the improvement process, the longest delay is associated with the time required to evaluate parents by the performance of their progeny, the so-called progeny test.

**In** tree species, 20 or more years elapse before final harvest, so genetic improvement from selection of progeny-tested parents is necessarily slow. Any technique that allowed early prediction of growth, or yield at final harvest, would be of major utility. For example, if yield could be predicted at 5 years instead of at 20 years, then 4 cycles of progeny testing and selection could be accomplished in the time usually required for one cycle, and genetic improvement could be 4 times as great. The higher intensity of selection possible with juvenile material compared to mature trees is an added benefit. The advantages of early testing have been discussed in detail by Nanson (1967, 1970).

*Developing a Concept of Growth*

The first step in developing a procedure for early progeny evaluation was to construct a conceptual framework or model to explain genetic variation in growth. Our approach was to maintain the simplest possible concepts in order to develop practical, operational techniques for predicting the relative superiority of progenies. Overly complex procedures would be impractical in large scale screening efforts. Once the problem has been satisfactorily attacked on an elementary level, more complex physiological analysis will be desirable.

Photosynthetic $CO_2$-uptake must be an important quantity in explaining growth, though demonstrations are rare. Total plant dry weight is very nearly a simple multiple of the weight of organic carbon metabolically assimilated as $CO_2$. The problem, however, lies in estimating $CO_2$-uptake. Two approaches have been used in the past: (1) The direct measurement of the rate of $CO_2$-uptake for a brief period and (2) the calculation of net assimilation rate over longer periods using the techniques of growth analysis. Both of these techniques are valuable.

$CO_2$-uptake is usually measured using infra-red gas analyzers under controlled conditions. But in the past, $CO_2$-uptake was only rarely correlated with current growth and no one was able to predict future growth from rates of $CO_2$-uptake. There were two reasons why so little success in explaining genetic differences in growth were achieved with this approach. The first was the failure to take either seasonal or ontogenetic changes in the rate of $CO_2$-uptake into account. Photosynthetic rate was implicitly treated as a constant. The

second reason was a failure to appreciate the profound multiplying effect of small differences in foliar growth. A small advantage in leaf surface will have a major effect on growth because the process of growth is analogous to that of compounding interest *(e.g.* Sweet and Wareing 1966).

The importance of seasonal change in the rate of $CO_2$-uptake and differences in the rate of leaf production was recognized by investigators using the techniques of growth analysis. Through periodic harvests and weighing of samples from an experimental population, the rate of increase in dry weight per unit leaf can be calculated for each sampling period, and the pattern of foliar increment can also be charted. The rate of increase in dry weight per unit leaf is called the net assimilation rate (NAR) and can be considered equal to net $CO_2$-uptake by the plant in the light, minus $CO_2$ loss in respiration in the dark, multiplied by a constant, which is the ratio of dry weight increment $g$-$ICO_2$ assimilated. Unfortunately, growth analysts never developed objective and integrative methods of comparing one genotype to another with respect to NAR. Within species of crop plants, Heath and Gregory (1938) and Watson (1952) found a great deal of variation in the rate of increase in leaf area and discounted relatively smaller differences in NAR. From the observations of this influential group on highly selected crop plants, the general belief developed that differences in NAR were unimportant in explaining growth or yield *(e.g.* Watson 1952, 1967); or what is the same thing, that differences in the rate of photosynthetic $CO_2$-uptake and respiration made little contribution to variation in yield. However, not all growth analysts felt that variation in NAR was unimportant.

There was obvious variation in NAR in wild, unselected populations of forest trees (Figure 1). In loblolly pine *(Pinus taeda* L.), there were not only differences in NAR but the rate changed during the year and the pattern of change differed among progenies (Ledig and Perry 1969). NAR in several progenies dropped rapidly starting shortly after germination, but in a few others it remained stable until cold weather in November. On anyone date, the rank of progenies for NAR (and presumably the rate of $CO_2$-uptake) would be very different from the rank on another date. It is no wonder that the rate of $CO_2$-uptake measured only once during the year would infrequently be correlated with growth.

For objective comparisons among progenies, Ledig and Perry (1969) used the integral or area under the curve of NAR over time, a seasonal summation of g dry weight increase g-1 leaf dry weight.
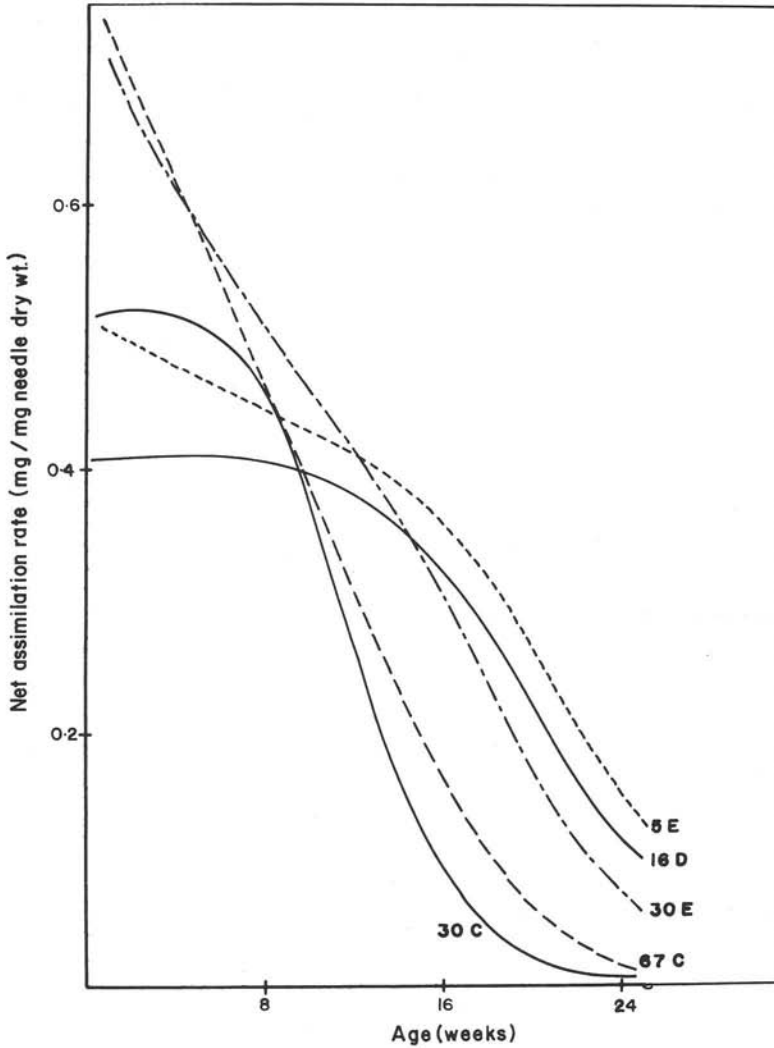
Figure 1. Net assimilation rate in 5 full-sib progenies of loblolly pine, showing the changes with age from germination. Each curve is the progeny of a different cross, identified by code.

The integral could be taken mathematically if the curve was described by a mathematical equation or planimetrically if the relationship was not. For 26 full-sib progenies of loblolly pine, the integral was strongly correlated with observed dry weight after 21 weeks of

growth. NAR explained 64 percent of the variation in growth ($r =$ 0.80). Such a correlation is, in part, fortuitous because it could easily have been obscured if the loblolly pine progenies had differed greatly in the rate at which they invested current photosynthate into new leaf growth. A favorable distribution of photosynthate to leaf growth can be a more important determinant of growth rate than the rate of photosynthetic $CO_2$-uptake itself. An example of this is Figure 2, which shows hypothetical growth curves generated by a simulation model. A slight superiority in leaf growth results in greater total dry weight than that obtained with higher rates of $CO_2$-uptake but less leaf growth.

The results of the loblolly pine study on seasonal patterns of NAR and the distribution of photosynthate suggested a simple model of growth:

$$Y(t) = K \int [aP(t) \bullet Y(t)^b \bullet H_L(t) - a'R_s(t)Y(t)^{b'} \bullet H_D(t) - a''24 \bullet R_r(t) \bullet Y(t)^{b''}]dt,$$

where:

$Y(t)$ = total dry weight,

$P(t)$ = net rate of $CO_2$-uptake in the light ($g^{-1}$ leaf dry weight),

$R_s(t)$, $R_r(t)$ = rates of respiration of the shoot in the dark ($g^{-1}$ shoot dry weight) and root respiration ($g^{-1}$ root dry weight),

$H_L(t)$, $H_D(t)$ = hours of light and dark,

$K$ = a constant, the ratio of dry weight increment $g^{-1}$ $CO_2$ assimilated, ca. 0.5.

$a, a'$ , $a''$, $b, b'$ ,$b''$ are allometric parameters relating leaf, shoot, and root dry weight to total dry weight; $i.e.$ $L(t) = aY(t)^b$, $S(t) = a' Y(t)^{b'}$, $RT(t) = a''Y(t)^{b''}$, where $L(t)$, $S(t)$, and $RT(t)$ are leaf, shoot, and root dry weight, respectively. In pines, the allometric parameters are apparently little influenced by environmental fluctuations and remain stable for at least several years (Ledig, Bormann, and Wenger 1970). However, alternate methods may be used to allocate photosynthate among organs. In use, the expression under the integral is summed iteratively, using a computer program. The rationale for the model was more fully explained elsewhere (Ledig 1969). Increasing complexity could be added by making $P$, $R_s$, and $R_r$ functions of light, temperature, and moisture stress as well as time. We have often used the model in a reduced form depending on the type of data available.

The objective in constructing the model must be borne in mind. It was constructed to discriminate among genotypes; $i.e.$ to *predict* genetic *differences* in growth when estimates of the parameters were provided, but not necessarily to estimate actual growth. Its
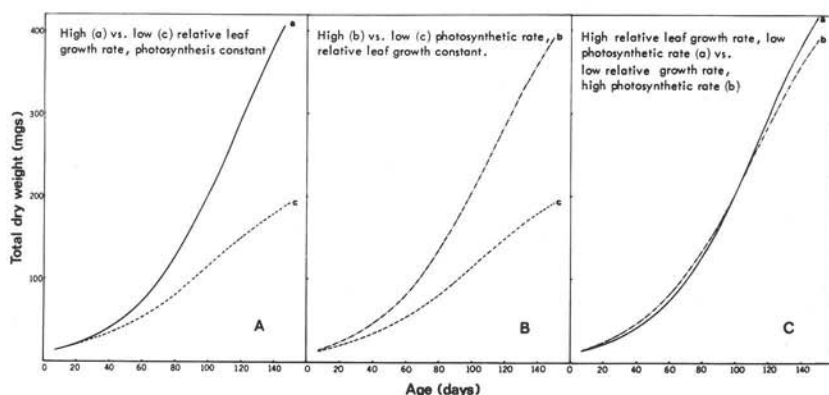
Figure 2. Hypothetical growth curves generated by simulation using a model similar to that described in the text and based on parameters observed in a study of loblolly pine seedlings. A. Situation in which photosynthetic rate is identical for *a* and *c* while distribution of current assimilate to leaf growth is 40 percent for *a* and 35 percent for *c*. B. Growth when distribution of assimilate is identical for *b* and *c* but the photosynthetic rate for *b* is 40 percent greater than that of *c*. C. Comparison in which both photosynthesis and the distribution of assimilate for growth differ; *b* distributes 35 percent of its assimilate to leaves while *a* distributes 40 percent to leaves and the rate of photosynthesis of *b* is 40 percent greater than that of *a*. Obviously, a higher photosynthetic rate does not ensure superior growth.

utility for the purpose depends first on whether *there is genetic variation* in 1) $CO_2$-exchange rates and in 2) allometry, the distribution of growth. For both photosynthetic and allometric parameters, there apparently is variation in wild plants. For example, in the study of NAR in loblolly pine referred to above, not only the rate but also the seasonal pattern differed among progenies (Ledig and Perry 1969). Direct measurements of $CO_2$-uptake also revealed a two-fold difference among progenies in the rate of photosynthesis and a large interaction with season of measurement (Ledig and Perry 1967). Genetic variation in the allometric coefficient, and therefore the distribution of growth to the leaves, was also demonstrated for loblolly pine (Ledig and Perry 1965).

The next task was to test the model. The test of the model involved two steps. First, did it adequately *describe* or *simulate* dry weight growth and secondly could it *predict* future growth based on parameters measured in the juvenile stage. It was much easier to test the descriptive powers of the model than its predictive powers.

*Simulating Growth*

In the first test of the growth concept, parameters for 26 full-sib progenies of loblolly pine were substituted in the model and growth was simulated. Net assimilation rates rather than $CO_2$-exchange rates were used as input. Allometric coefficients were determined by iterative regressions of leaf on total dry weight. The correlation between dry weight simulated by the model and actual observed dry weight at 21 weeks was r = 0.97 (Ledig 1969). The model explained 94 percent of the observed variation in growth while the seasonal integral of NAR had explained only 64 percent. Use of the model provided a substantial increase in precision in explaining genetic differences in growth.

In a second test, 3 species of larch, European larch *(Larix decidua* Mill.),Japanese larch (L. *leptolepis* [Sieb. et Zucc.] Gord.), and Siberian larch (L. *sibirica* Ledb.), were observed for two growing seasons. Seedlings were grown in pots, sunken in the soil, but were maintained outdoors with no other environmental manipulation. Photosynthetic $CO_2$-uptake, $CO_2$-emission in respiration, and the distribution of growth to the leaves was measured at intervals during the two years. Rates of $CO_2$-exchange were measured at temperatures corresponding to the long-time mean maximum temperature for each period. Though measurements were infrequent, the larches apparently differed in their seasonal pattern of photosynthetic $CO_2$-uptake as seen in Figure 3 (Ledig and Botkin 1974). The rates were used as input to the model, assuming that they changed linearly between measurement periods. Terms pertaining to root respiration were omitted. Ranks for simulated growth and for observed dry weight at the end of the second growing season were almost perfectly correlated (Figure 4). From these two examples, it may be concluded that the model adequately simulates differences in growth.

The simple methods of cut and weigh required for growth analysis and the more sophisticated methods of $CO_2$-exchange measurement requiring elaborate instrumentation both enabled satisfactory predictions of differences in dry weight growth when appropriate forms of the model were used. However, there are potent advantages to the development of $CO_2$-exchange rate technique. From measurement of $CO_2$-exchange, the influence of photosynthetic $CO_2$-uptake can be separated from that of respiration and the contribution of various organs to total respiration can be partitioned. It may become
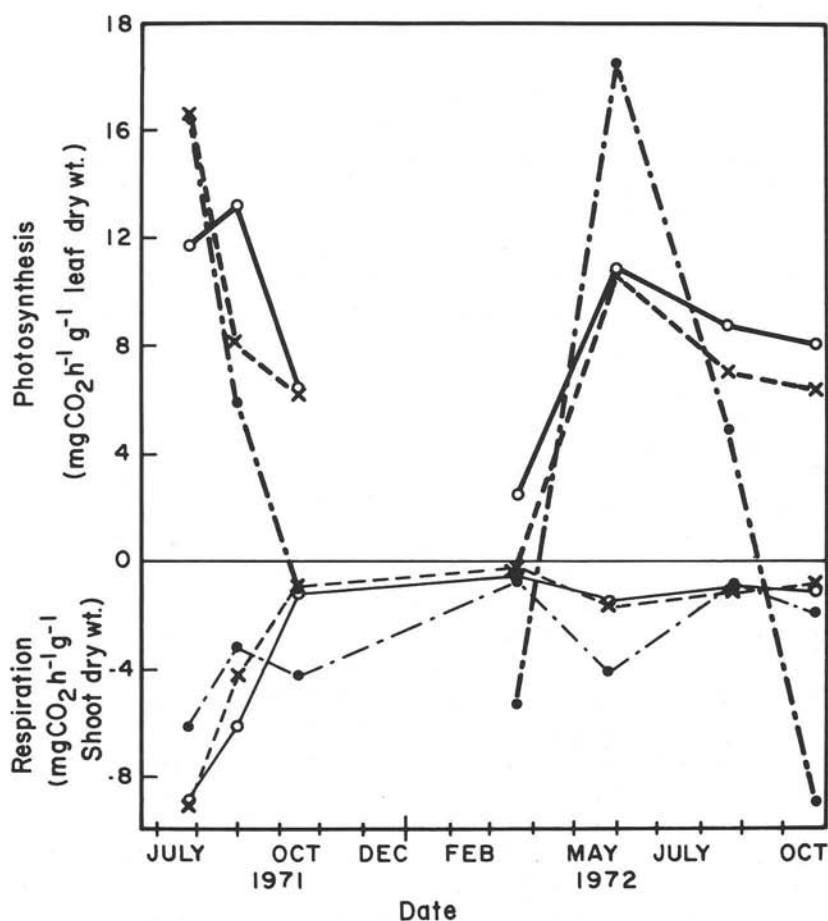
Figure 3. Seasonal patterns of photosynthetic $CO_2$-uptake (heavy lines) and respiratory $CO_2$-emission (light lines) in three species of larch during their first two growing seasons; 0 – European larch, • – Siberian larch, X – Japanese larch. Seedlings were leafless from late October to March.

desirable to breed for each of these components. Furthermore, $CO_2$-exchange rates *per se* are only a first step in understanding the growth process. Differences in the rate of $CO_2$-uptake may result from differences in the resistance to transfer of $CO_2$ through the boundary layer, the stomata, and the mesophyll, or from differences in nature of the carboxylating enzymes, rates of Hill reaction, processes of photorespiration, rate of translocation, or a myriad of other
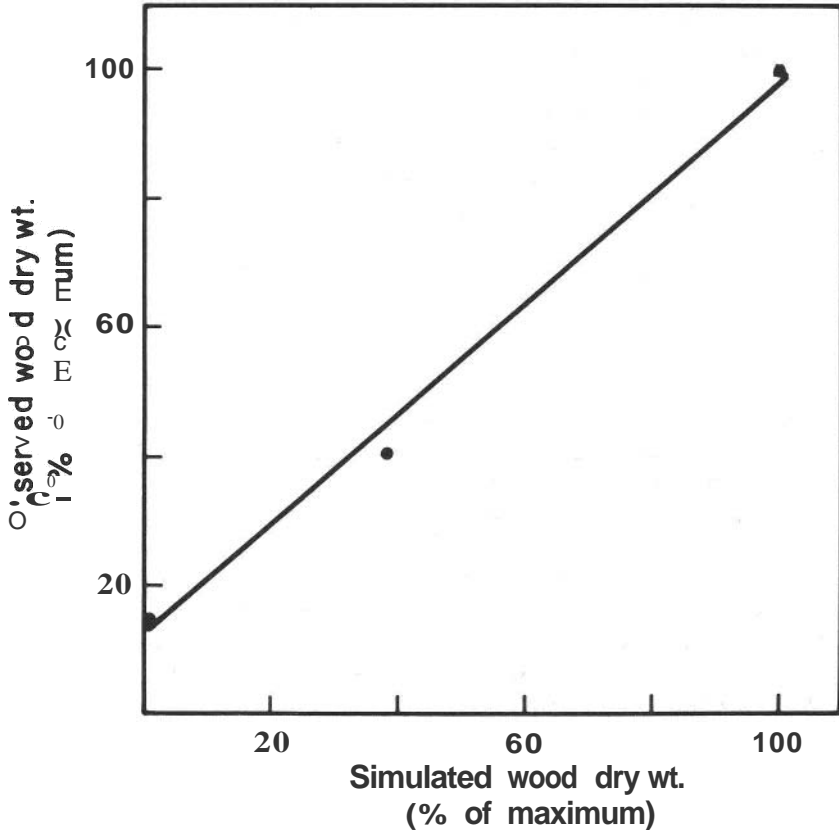
Figure 4. Relationship between actual observed wood dry weight of three larch species after two growing seasons and that simulated using rates of $CO_2$-exchange and the distribution of growth among leaves, stem, and roots. Values as percent of maximum for each scale.

factors. These characteristics can be measured using gas exchange and other physiological techniques.

The growth model has utility in its present simple form but it also has the capacity for expansion to incorporate more physiological complexity. Ultimately the breeder will wish to modify component processes or at least prevent the fixation of genes with negative value. Selection for the compound trait, yield, in agronomic plants has apparently succeeded by increasing the rate of leaf area expansion, but in the process, the high photosynthetic rate of some primitive forms has been inadvertently sacrificed (Evans and Dunstone

1970). Could high rates of leaf expansion have been combined with high rates of $CO_2$-uptake dm$_{-2}$ if the components of yield had been treated individually?

There is another advantage of measuring $CO_2$-uptake rather than using growth analysis. Growth analysis requires large numbers of plants because they must be destructively harvested at each measurement period. Single seedlings can provide several branches to permit measurements of $CO_2$-exchange over extended periods.

*Predicting Growth*

The next step in testing the utility of the model is to determine whether it can predict. growth to harvest age using parameters measured during the seedling stage. To follow an experiment from seedling to harvest age could require a period longer than the professionallife of a single investigator. There is an alternative. Progeny tests in forest trees were initiated on a large scale in the last decade and some of these are now old enough to provide a good indication of mature size. In some cases, seed of the original crosses is available in storage and in most cases, the parents still survive so the cross could be remade. It is possible to measure parameters of $CO_2$-exchange and the distribution of photosynthate on seedlings of these crosses, extrapolate growth using the model, and correlate results of the extrapolation with the performance of the older siblings in the advanced progeny tests. Such a test will be started in spring 1974. Successful extrapolation will depend on consistent phenological patterns of $CO_2$-exchange and distribution of photosynthate from year to year.

*Utilizing Measurements of Photosynthetic Rate* in *Tree Improvement*

How can the concepts presented above be used in forestry? The question may be premature, but I believe the measurement of photosynthetic $CO_2$-uptake already has utility. There is now sufficient verification of the role of photosynthesis arid the distribution of photosynthate in growth to permit the selection of provenances and

progenies for specified climatic conditions and to perhaps permit the evaluation of hybrids.

One benefit of the model was to show that the rate of photosynthetic $CO_2$-uptake could indeed be related to growth when the effect of relative leaf growth was taken into account. That demonstration was analogous to the breaking of the 4-minute mile in track; once the possibility was demonstrated, it became apparently simpler to repeat. Much of our recent work has utilized photosynthetic rate to explain differences in growth and survival among wild populations.

*Testing Provenances.* Balsam fir *(Abies balsamea* [L.] Mill.) provided an example of how photosynthetic rate might be used in provenance selection. A provenance is the geographical source of a seed lot; *i.e.* its place of origin, in which its genome has been molded by natural selection and other population processes. The term is simultaneously used to designate a population originating from a specific locality. A major problem in commercial forest management is to choose the right provenance as a source of seed for reforestation. One aspect of the problem is to determine whether gains can be made by using seed of non-local provenance when a choice is available. The other side of the coin is to delimit seed collection zones, zones in which seed can be safely exchanged when it is necessary, without losses in survival or growth.

Photosynthetic response to temperature, light, and moisture stress could be used to determine the range of adaptation of a provenance and its suitability for a particular environmental regime. For example, Fryer and Ledig (1972) grew balsam fir seedlings representing populations native to different elevations on Mt. Moosilauke, New Hampshire under uniform conditions in a greenhouse with mean diurnal temperature of $27°C$. The temperature at which the rate of $CO_2$-uptake is maximum, the temperature optimum, was determined for each provenance. The optimum temperature for photosynthetic $CO_2$-uptake for high elevation provenances from the cool upland was lower than the optima for low elevation provenances from the warmer lowland, indicating that these populations were adjusted to their temperature environment by natural selection. The relationship between elevation and the temperature optimum for photosynthetic $CO_2$-uptake was very close (Figure 5). Even small transfers of 500 feet in elevation might have noticeable effects on productivity in such finely attuned populations.

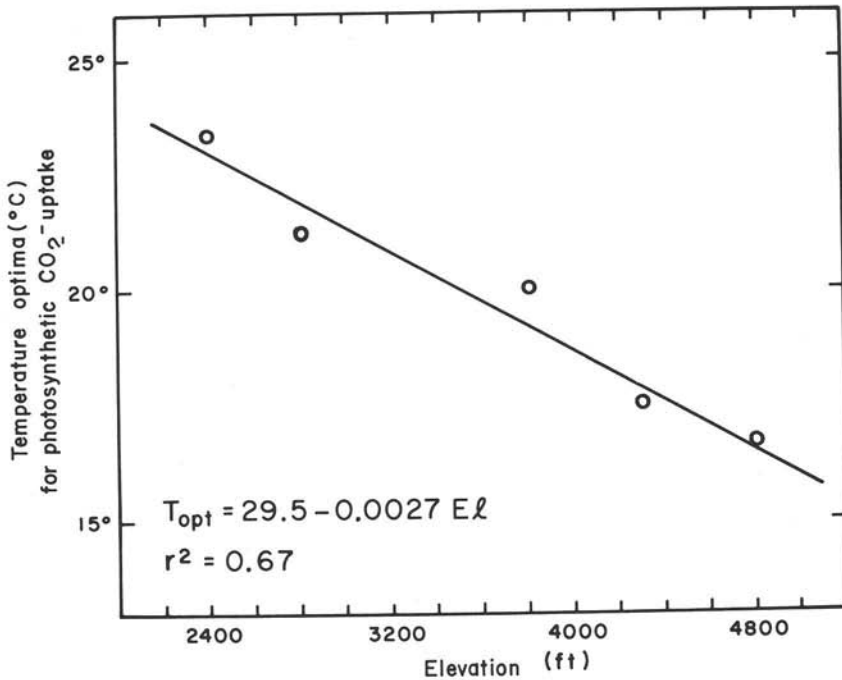From the balsam fir experiment, we can try to answer the ques-

Figure 5. Relationship of temperature optimum for net photosynthetic $CO_2$-uptake with elevational origin of balsam fir seedlings.

tion: What is the best choice of provenance to maximize growth in an environment with mean temperature of 27°C? The rate of photosynthetic $CO_2$-uptake at 27°C was interpolated from the mean temperature response curves (Figure 6A). The lowest elevation provenance had the highest rate of $CO_2$-uptake at 27°C, which is not surprising because at 2400 ft msl in the White Mountains, the summertime daily maximum approaches 27°C while temperatures at higher elevations deviate progressively from this value. The correlation between dry weight growth and the interpolated rate of $CO_2$-uptake at 27°C was r = 0.86 (Figure 6B). A correlation of this magnitude with volume yield at rotation age would be more than enough to favor early selection over late selection, but lack of correlations should not be unexpected. Only a scheme such as the model reviewed here, taking into account allometric relationships and seasonal changes in $CO_2$-uptake, will provide consistent correlations with growth. High correlations of growth with rate of $CO_2$-uptake in a
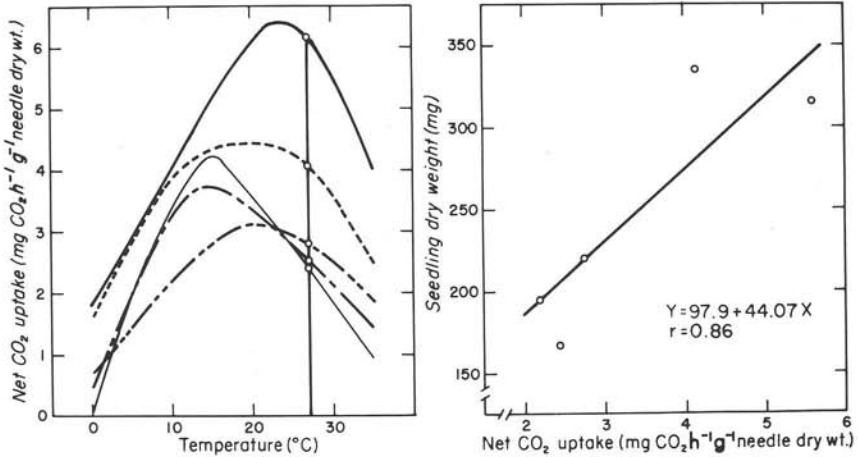
Figure 6. A. Net rates of $CO_2$-uptake of balsam fir from different elevational zones as a function of temperature. B. Relationship between observed seedling dry weight and rates of photosynthetic $CO_2$-uptake interpolated from A. Points represent values interpolated at 27°C.

particular instance merely means that there is little variability in the other components of growth.

The temperature response curves are not sufficient to suggest which provenance should be selected for temperature regimes other than 27°C. The response curves depend on the thermal history of the plants and both shape and magnitude of the photosynthetic response will change under other conditions. However, seedlings could be grown under any desired environmental regime and their response tested in the manner described here.

*Evaluating Species and Hybrids.* An example in which photosynthetic rate and growth depend on the interaction between genotype and environment was illustrated by a recent study of black and red spruce (*Picea mariana* [Mill.] B.S.P. and *P. rubens* Sarg.) and their hybrids from the Canadian Maritimes (Manley and Ledig, in preparation). Using a hybrid index (Manley 1971), individuals were chosen that corresponded to pure species (0 and 100 respectively on the hybrid index) to intermediate hybrids (50), and to backcrosses of the hybrid to both parental types (25 and 75). These individuals were then intercrossed to produce 9 classes of progeny grading from pure black spruce to pure red spruce (0, 12.5, 25, 37.5, 50, 62.5, 75, 87.5, 100).

Black spruce had distinctly higher rates of $CO_2$-uptake than red spruce when grown at low temperatures (Figure 7B,D), and red spruce tended to have a higher photosynthetic rate than black spruce when grown at low light and high temperature (Figure 7A,C). Other experiments indicated that photosynthesis in red spruce was light saturated at about 1000 ft-c while black spruce was capable of responding to light intensities at least 3 times as great. While red spruce had a higher rate of $CO_2$-uptake than black spruce at low light, the reverse was true at high light.

These responses seem reasonable with respect to the habitats in which these species are normally encountered. Black spruce is characteristic of bogs which are sinks for cold air, while red spruce occurs on the warmer uplands. Red spruce is capable of maintaining itself in deep shade while black spruce is an early seral species typical of logged or otherwise exposed sites. Black spruce fails rapidly under a closed forest canopy on the warmer uplands but succeeds itself in the cooler bogs. The results of $CO_2$-exchange provide a physiological rationale for the ecological observations, and should guide decisions on reforestation and silviculture of these species.

However, the hybrids were the most interesting aspect of the study. Under *all* conditions the hybrids were inferior to one or the other parent. **In** fact, they exhibited negative heterosis; *i.e.* rates of $CO_2$-uptake in the hybrids were less than the mid-parental mean, or in many conditions, even less than the mean of the poorer parent (Figure 8). The further the value of the hybrid index from those of either pure parental species, the poorer were the hybrid derivatives. Under all conditions, growth of the 9 groups were correlated with their rate of photosynthetic $CO_2$-uptake under the same conditions; the correlation coefficients ranged from 0.45 to 0.83. Again, correlations of this magnitude with volume yield at rotation would be sufficient to favor early selection over late selection.

Usually hybrids are expected to have special vigor. **In** fact, Canadian forest geneticists suggested that hybrids between black and red spruce should be produced for reforestation. The high hybrid mortality occasionally observed in nursery beds was largely ignored and it is now apparent that it was given insufficient weight in evaluating the potential of the hybrid. Therefore, this study had practical significance. The results suggest that hybridization between the two species should be avoided, for there seems to be no condition under which one or the other parent is not superior, even to recurrent backcrosses which resemble the parent species closely. Particular
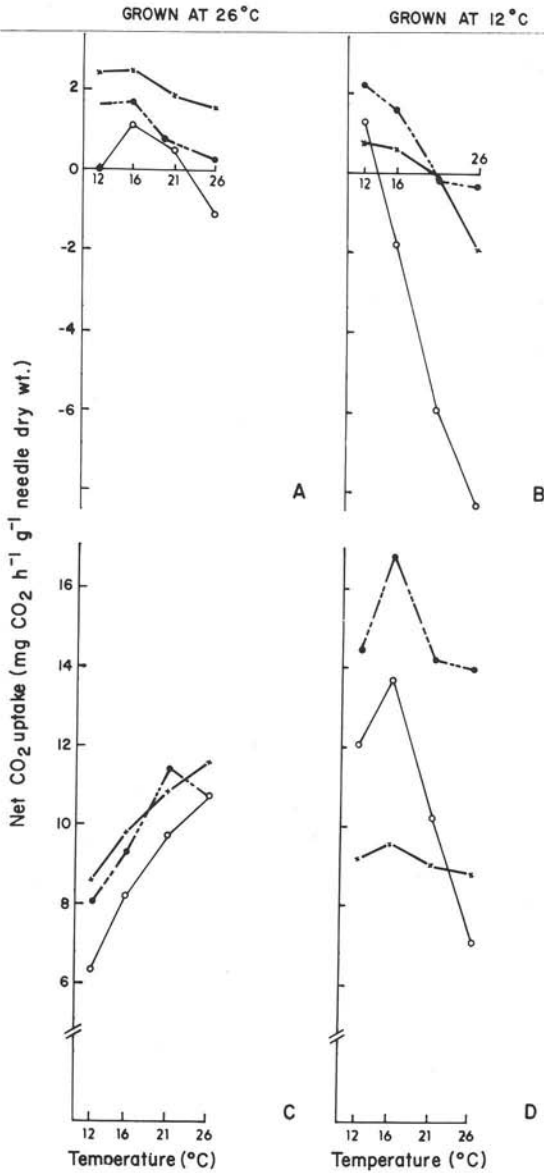
Figure 7. Rate of photosynthetic $CO_2$-uptake in relation to temperature for black (•)
and red (X) spruce and their hybrid (O) grown at 100 ft-c and either 12°C or 26°C.
A-B measured at 100 ft - c; C-D measured at 2000 ft - c. Note the strong interaction
of species with both growth regime and measurement conditions and the general
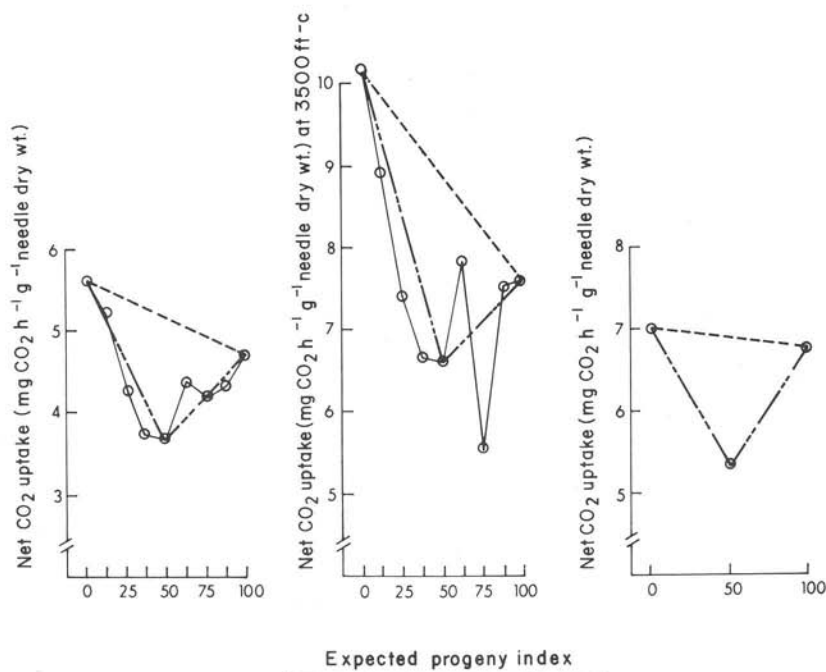inferiority of the hybrid.

Figure 8. Rate of photosynthetic $CO_2$-uptake in three separate experiments plotted over a progeny index grading from black spruce to red spruce through hybrid derivatives. Dashed line represents expected value for hybrids for a completely additive model of inheritance. Another line connects the $F_1$ hybrid with parental species. In all cases the hybrid exhibits negative heterosis and the negative deviation of hybrid derivatives apparently increases the further their hybrid index deviates from that of the pure parental species. Even recurrent backcrosses (12.5 and 87.5) fall below expectations based on the additive model.

care should be taken to isolate seed orchards of black and red spruce respectively from contamination by wild pollen of the other taxon.

*Family Selection.* The ultimate goal in the measurement of photosynthetic rate will be to choose families with superior potential for growth. Early results were promising. Three half-sib families (seed from known mother-trees with random pollen parents) of sycamore (*Platanus occidentalis* L.) were grown outdoors for two years, exposed to ambient conditions. Photosynthetic rate and the distribution of photosynthate were measured periodically and growth was simulated as outlined above for larch (Ledig and Botkin 1974). Actu-

al and simulated dry weights deviated considerably because of the failure to account for root respiration. The same caution applies to the larch study discussed above. However, the agreement between rank based on model simulations of growth and rank based on observed dry weight after two years was nearly perfect (Figure 9). The objective of the model (*i.e.* to simulate *differences* in growth) was fulfilled.
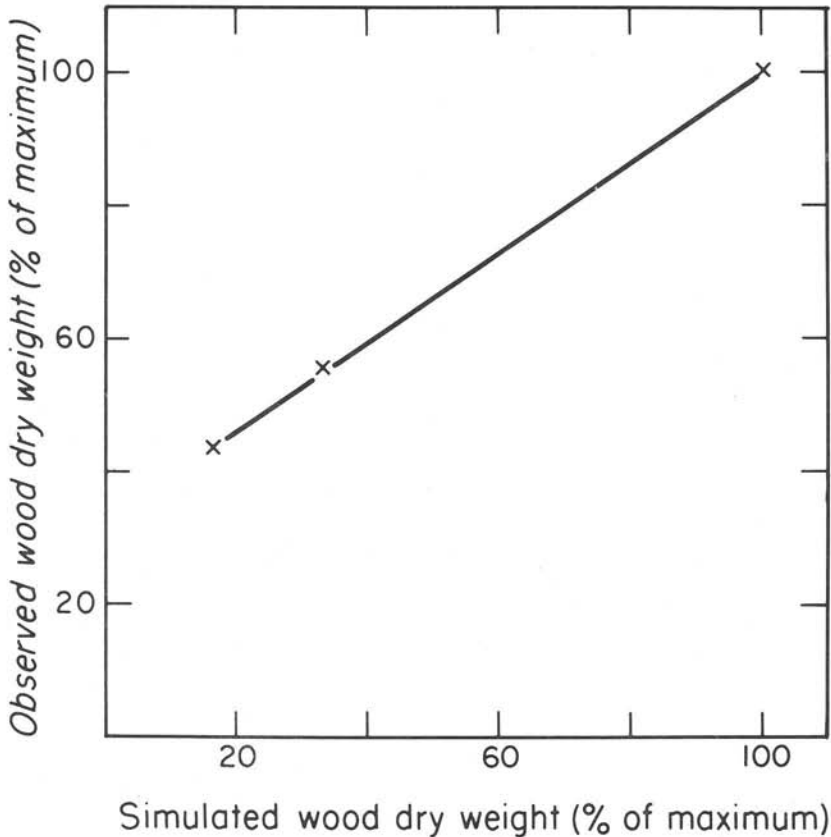


Figure 9. Relationship between actual observed dry weight of three half-sib progenies of sycamore after two growing seasons and dry weight simulated using rates of $CO_2$-exchange and the distribution of growth among leaves, stem, and roots. Values are percent of maximum for each scale.

*Conclusion*

The research reviewed here demonstrated that growth can be related to photosynthetic rate in tree seedlings. Photosynthetic rate can be used to judge adaptation to specified environmental conditions. The *combination* of photosynthetic and respiratory rates with parameters expressing distribution of photosynthate to leaves, stem, and roots, can be used to *simulate* genetic differences in growth almost perfectly. But, can physiological parameters at the juvenile stage be used to *predict* differences in growth, enabling early selection and shortening the breeding cycle? Intuitively, I think the answer is yes, if seasonal patterns of photosynthesis and of the distribution of photosynthate remain consistent from year to year as seedlings age. The final test of these concepts will be complete when simulations based on seedling parameters are extrapolated to harvest age and compared with actual yield of mature trees in the field.

Even if the goal of early prediction based on juvenile physiology is not achieved, physiological characteristics might be a valuable aid to selection when combined with field tests on older seedlings or saplings. The increased understanding of the components of yield is itself an important benefit of the research. In breeding programs both for agronomic and forest crops, selections for high yielding families or varieties may result in the loss of individuals that grow less well but possess some desirable genes. Superior types might be produced by crossing individuals to achieve new combinations of characteristics. To illustrate the potential, the growth model was used to simulate hypothetical loblolly pine with different combinations of $CO_2$-uptake rate, seasonal patterns of $CO_2$-uptake, and allometric parameters at high, middle, or low levels spanning the range observed during the course of these investigations. Some of the combinations grew much more rapidly than any loblolly pine actually observed (Ledig 1969). The hypothetical growth curves suggest that if it is physiologically and genetically possible to recombine high rates of photosynthesis with a long seasonal duration and a high initial distribution of growth to leaves, breeders might achieve greater gains than any now anticipated.

Continued reliance on visual selection alone will fail to provide the gains that could be achieved by developing our knowledge of the components of yield in order to engineer new, more productive combinations of these characteristics. Such paths might lead to new

highs of production through adaptive valleys uncrossable by the usual procedures of selection for the compound characteristic, yield. It is a path worth exploring.

# LITERATURE CITED

Evans, L. T. and R. L. Dunstone. 1970. Some physiological aspects of evolution in wheat. Austral. J. BioI. Sci. 23: 725-741.

Fryer, J. H. and F. T. Ledig. 1972. Microevolution of the photosynthetic temperature optimum in relation to the elevational complex gradient. Can. J. Bot. 50: 1231-1235.

Heath, O. V. S. and F. G. Gregory. 1938. The constancy of mean net assimilation rate and its ecological importance. Ann. Bot. N. S. 2: 811-818.

Ledig, F. T. 1969. A growth model for tree seedlings based on the rate of photosynthesis and the distribution of photosynthate. Photosynthetica 3: 263-275.

Ledig, F. T., F. H. Bormann, and K. F. Wenger. 1970. The distribution of dry matter growth between shoot and roots in loblolly pine. Bot. Gaz. 131: 349-359.

Ledig, F. T. and D. B. Botkin. 1974. Photosynthetic $CO_2$-uptake and the distribution of photosynthate as related to the growth of larch and sycamore progenies. Silvae Genet. 23. In press.

Ledig, F. T. and T. O. Perry. 1965. Physiological genetics of the shoot-root ratio, p. 29-43. *In* Proc. Soc. Am. Foresters, 1965. Detroit, Michigan.

Ledig, F. T. and T. O. Perry. 1967. Variation in photosynthesis and respiration among loblolly pine progenies, p. 120-128. *In* Proc. Ninth Southern Conf. on For. Tree Improvement. Knoxville, Tennessee.

Ledig, F. T. and T. O. Perry. 1969. Net assimilation rate and growth in loblolly pine seedlings. For. Sci. 15: 431-438.

Manley, S. A. M. 1971. Identification of red, black and hybrid spruces. Dept. Environ., Can. For. Service Pub. No. 1301. Ottawa. 14 p.

Nanson, A. 1967. Modele theorique pour l'etude des tests precoces [in French, English summary]. Biometrie-Praximetrie 8: 84-107.

Nanson, A. 1970. Juvenile and correlated trait selection and its effect on selection programs, p. 17-26. *In* Papers presented at the Second Meeting of the Working Group on Quantitative Genet., Section 22 IUFRO. Raleigh, North Carolina.

Sweet, G. B. and P. F. Wareing. 1966. The relative growth rates of large and small seedlings in forest tree species, p. 110-117. *In*

Forestry Supplement, Physiology in Forestry. Oxford University Press.

Watson, D. J. 1952. The physiological basis of variation in yield. Advan. in Agron. 4: 101-145.

Watson, D. J. 1967. Physiological characteristics of the growth of sugar beet crops on different soils. 1.I.R.B. 2: 226-231.

# THE POTENTIAL OF *IN VITRO* TECHNIQUES FOR FOREST GENETICS

Peter S. Carlson₁ and Thomas B. Rice'
Biology Department,
Brookhaven National Laboratory,
Upton, New York 11973

## Introduction

This short paper will outline how newly developed techniques involving the *in vitro* culture of plant cells might be used in forest genetics. It will be of a speculative nature because the authors are not forest geneticists, and very few of the techniques outlined in this review are now applicable to trees. However, there is every reason to expect that once current technical limitations are overcome, trees can be manipulated *in vitro* like other higher plants.

A forest geneticist works with very difficult material. The life cycle of a tree is a long process and the sexually mature organism is quite large, so that the use of standard Mendelian manipulations is not possible, or at best, difficult. There is extensive natural variation in tree populations so that generating genetically uniform starting material is an arduous task. Furthermore, many of the characteristics of interest to the forest geneticist are not transmitted by single genes, but are determined by polygenic systems and are analyzable only with the use of large populations and extensive quantitative measurements (Libby *et at.* 1969, Hattemer 1963). Use of *in vitro* techniques would help circumvent these difficulties, thereby allowing a more complete genetic analysis of forest trees and efficient utilization of genetic variants in practical breeding programs.

The potential of using cell culture methods for genetic analysis is dependent upon the ability to carry out certain manipulations *in vitro*. Unfortunately, the technology for handling cells of trees in

'Present Address: Department of Crop and Soil Sciences, Michigan State University, East Lansing, Michigan 48823

culture has not developed as rapidly as for certain other higher plants such as tobacco (Linsmaier and Skoog 1965, Gibbs and Dougall 1965, Vasil and Hildebrandt 1965, Nagata and Takebe 1971, Nakata and Tanaka 1968, Tanaka and Nakata 1969, Carlson 1970, Carlson *et al.* 1972). There is no reason to expect that the problems are not primarily of a technical nature, and it is anticipated that *in vitro* techniques can be extended to trees with further work. This paper will point out the technical manipulations necessary to accomplish a given experimental goal, and will indicate generally what has been accomplished with tree tissue cultivated *in vitro.* Several techniques which illustrate the potential of *in vitro* cell cultures will be discussed. Many *in vitro* techniques are of importance to forest biochemists and physiologists, but these possibilities will not be considered here.

## IN VITRO *Cloning and the Rapid Propagation of Genotypes*

In several species of higher plants, large genetically uniform populations of individuals can be regenerated from cell suspensions ultimately derived from a single cell. Such *in vitro* cloning would be commercially important for the rapid propagation of superior individuals, and is essential for species which are difficult to propagate vegetatively by the usual methods. It would also be useful for the geneticist in generating uniform populations with which to analyze an important trait or the response of a genotype to different environments. In addition, the steps required for this technique are essential for other *in vitro* methods discussed below.

Three basic manipulations are required for cloning. First, it is essential that cells isolated from the tree be induced to divide in a rapid unorganized fashion. The resulting undifferentiated proliferation, termed a callus, can be induced from a variety of tissues in many plant species by manipulating hormone levels in the culture medium (Street 1973). Callus growth has been initiated from the cambial and meristematic regions of many woody species. In most early reports, sustained growth of callus tissue was achieved only when coconut milk or other undefined organic supplements were added to the culture media (Gautheret 1959, Pelet *et al.* 1960). Tissues from green ash, *(Fraxinus pennsylvanica* Marsh.), coffee *(Coffea arabica* L.), tea *(Thea sinensis* L.), redwood *(Sequoia sempervirens* [D. Don]

Endl.), white spruce (*Picea glauca* [Moench] Voss), western white pine (*Pinus monticola* Dougl.), and Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) have now been cultured on chemically defined media (Wolter and Skoog 1966, Staritsky 1970, Ogutuga and Northcote 1970, Ball 1950, Reinert and White 1956, Harvey 1967, Brown and Lawrence 1968, Winton 1972). The use of defined media is not required for cloning, but it enhances the applicability of the technique to genetic and physiological problems. Second, it is essential that cells of the callus be dispersed and grown in liquid medium to generate a suspension culture consisting of single cells or small clumps of cells. This step is routine with a number of higher plant species (Steward *et al.* 1969), and has also been achieved with tissue from a few woody species (Winton 1972, Becker *et al.* 1964, Mathes 1964). The third and final step is to complete the cycle by regenerating entire plants from the single cells or small clumps. This is normally accomplished by manipulating hormone levels in the culture medium. In general, plantlets develop from undifferentiated cells along one of two pathways. Cells in suspension culture may be stimulated to undergo organization directly into a plantlet through stages that resemble normal embryogenesis. Carrot is an example of a species that will form a plant directly from cells in culture (Steward *et al.* 1966). Alternatively, as illustrated by tobacco, cells from suspension are allowed to proliferate prior to applying the stimulus for shoot and root regeneration. Hence, organ differentiation occurs within a large cell mass (Skoog and Miller 1957).

Regeneration of plants is not a routine procedure with trees. To our knowledge there are no reports of organ formation by cells from suspension cultures. Considering the significance of these manipulations, heavy emphasis should be placed on their development. There is cause for optimism that this potential can be realized, since differentiation of shoots by callus tissues of redwood, European aspen (*Populus tremula* L.), quaking aspen (*Populus tremuloides* Michx.), eucalyptus (*Eucalyptus citriodora* Hook.) and coffee has been reported (Ball 1950, Mathes 1964, Wolter 1968, Winton 1968, 1971, Aneja and Atal 1969, Staritsky 1970). In each case the yield of buds was low and most authors had difficulty maintaining the growth of newly differentiated shoots. But Winton (1970, 1971) has grown young aspen trees in soil from callus-derived plantlets. Thus, despite present technical limitations, cloning will almost certainly be the first *in vitro* technique available for use by forest geneticists and breeders.

*Extending the Range of Genetic Variability Via Induced Mutations*

   Defined mutant types can be generated and recovered from cells of higher plants cultured *in vitro* (Chaleff and Carlson 1974). Cell cultures offer a microbial-like genetic system because it is possible to examine large numbers of individuals under defined conditions, and it is often possible to work with haploid cell lines. Mutant selection is an extension of *in vitro* cloning. The only additional steps required are mutagenesis of cell suspensions and application of mutant selection screens on regenerating tissue. These manipulations should not present many difficulties once methods for cloning have been defined. Cells in suspension can easily be exposed to the same spectrum of chemical mutagens that have been effective in microbial systems. The key to an effective selective system is the definition of conditions where only cells with the desired mutant phenotype can survive and proliferate. The following examples suggest some of the types of mutants which might be recovered using *in vitro* cultures.
*Trees Resistant to a Pathogen.* These would be recovered by selecting cells resistant to a toxin produced by the pathogen. The approach has been successfully applied in tobacco. By screening for mutants resistant to an analogue of the bacterial toxin, it was possible to recover mutants less susceptible to wildfire disease (Carlson 1973). Another approach would be to select for cultures which will not support growth of the pathogen *in vitro.* Problems associated with dual tissue culture of plant pathogens and host somatic cells have been discussed by Ingram (1973). *In vitro* expression of parasite resistance has been observed in several systems (Ingram 1967, 1973, Helgeson *et af.* 1972). Among woody species, Harvey and Grasham (1969) cultured blister rust fungus on cambial callus cultures of pine, but under their conditions infection of callus derived from a nonhost species was also observed (Harvey and Grasham 1971).
*Trees Resistant to Extreme Environmental Conditions. In vitro* methods should allow the selection of mutants resistant to a number of environmental perturbations. These mutants could be recovered by selecting for cells resistant to air pollutants such as ozone, sulfur dioxide, or hydrogen fluoride. Cells might be selected that were able to survive and grow on stressful agents such as NaCI or heavy metals. **It** may even be possible to use *in vitro* methods to select for trees able to grow under warmer, colder, or drier conditions by defining

the proper selective conditions *in vitro*. Certainly many additional mutant types of interest to the forest geneticist can be recovered from cell cultures.

### Extending the Range of Genetic Variability Via Somatic Hybridization

It has recently been demonstrated that an interspecific hybrid individual can be produced utilizing methods that do not involve standard sexual mechanisms (Carlson *et al.* 1972). In this work, somatic cells were treated operationally like gametes. Protoplasts were isolated from different tobacco species, stimulated to fuse, grown under restrictive conditions that permitted proliferation of hybrid cells only, and then regenerated into whole plants. Therefore, the technique required methods for isolating, fusing, and regenerating protoplasts in addition to procedures borrowed from the cloning and mutant isolation techniques. It is now a relatively straightforward matter to isolate large numbers of protoplasts from a wide range of higher plant species and to stimulate them to undergo fusion (Power *et af.* 1970, Cocking 1972, Keller *et af.* 1973, Withers 1973). Regenerating the cell wall around the protoplasts and stimulating their division is a more difficult manipulation and would almost certainly be the most difficult step to accomplish with trees. Selective recovery of hybrid fusion products from a mixed population that also contains homoplasmic fusion products and unfused cells presents another technical difficulty. In mammalian systems, selective recovery is achieved by utilizing parental cell lines that carry complementing auxotrophic or drug resistance mutations (Littlefield 1964). The use of recessive nuclear albino mutations has been suggested for this purpose in higher plant systems (Keller and Melchers 1973).

Since we are only beginning to explore the technique of protoplast fusion, it is difficult to imagine the limits to hybridization by this method and its full potential usefulness. Somatic hybridization bypasses the incompatibility barriers that serve to reproductively isolate many distantly related species. Thus, it is certainly expected that wider crosses should be possible than can now be accomplished by using standard sexual methods. Moreover, since the important feature of a forest species is its vegetative growth, this technique could be extended to interspecific hybrids that are not meiotically stable

(and hence infertile) by using *in vitro* cloning procedures for maintenance and propagation of the new varieties.

The successful hybridization of somatic cells also introduces the possibility of developing a parasexual genetic system in higher plant tissue cultures. Such a system would permit genetic analysis *in vitro,* thus bypassing the long generation times of forest species. Fusion alone is sufficient for analyzing genetic complementation. Completion of a parasexual system requires somatic recombination and resolution of the hybrid genome into its component parts. Somatic recombination has already been observed and characterized in whole plants (Vig and Paddock 1968), and should occur in cultured cells as well. Resolution of the hybrid genome requires a method to select for cells in which induced or spontaneous chromosome loss has occurred. The recent report that parafluorophenylalanine can be used to preferentially maintain the haploid state in higher plant cells in culture (Gupta and Carlson 1972) indicates that progress in this direction is being made. Thus, there is reason to hope that in the near future an *in vitro* genetic system will be available to the forest geneticist.

## *Induction of Haploids Via Anther Culture*

Haploid plantlets or callus tissue can be obtained in a number of genera using the technique of anther culture (Sunderland 1971). Within the immature pollen of cultured anthers, it is the vegetative cell that divides to generate an embryoid or an undifferentiated callus mass. Unfortunately, the relevant parameters are not understood, so that with the exceptions of a few Solanaceous species, haploid induction is a very rare event. However, the technique should be applicable to trees. In view of the fact that the conifer, gingko *(Gingko biloba* L.), was one of the first species in which haploid callus was derived from pollen (Tulecke 1957), there has been remarkably little continued effort in this direction. A variety of experimental procedures that induce parthenogenetic development also generate haploid material in many higher plants (Kimber and Riley 1963). Haploid white poplar *(Populus alba* L.) and aspen seedlings developed from seeds that were produced by flowers fertilized with experimentally inactivated pollen (Kopecky 1960, Winton and Einspahr 1968). Haploid tissue from any source would be very useful to

the forest geneticist. Haploid material can be readily diploidized to obtain isogenic stocks for breeding purposes, and haploid cell lines are very important because they permit direct selection for recessive mutant phenotypes.

## Summary

Forest species constitute an important resource that has not yet been domesticated by man. Forest populations thus contain extensive unselected natural variation. **In** this paper we have discussed *in vitro* techniques that would facilitate the characterization and analysis of natural genetic variants; techniques for the induction and isolation of new genetic variants; and techniques that should permit novel associations of genetic potential to engineer new cultivars uniquely adapted to man's needs. It should be reemphasized, however, that at the present time not one of these techniques is a routine procedure. Before the *in vitro* approach can be efficiently utilized it will also be essential that important characteristics of the mature tree (whether in terms of yield, product quality, or management requirements) be correlated with traits that can be measured in seedlings or with biochemical events that can be selected *in vitro*.

# LITERATURE CITED

Aneja, S. and C. K. Atal. 1969. Plantlet formation in tissue cultures from lignotubers of *Eucalyptus citriodora* Hook. Curr. Sci. (India) 38: 69.

Ball, K 1950. Differentiation in a callus culture of *Sequoia sempervirens.* Growth 14: 295-325.

Becker, G. K, P. A. HUi, and P. Albersheim. 1964. Synthesis of extracellular polysaccharide by suspensions of *Acer pseudoplatanus* cells. Plant Physiol. 39: 913-920.

Brown, C. L. and R. H. Lawrence. 1968. Culture of pine callus on a defined medium. For. Sci. 14: 62-64.

Carlson, P. S. 1970. Induction and isolation of auxotrophic mutants in somatic cell cultures of *Nicotiana tabacum.* Science 168: 487-489.

Carlson, P. S. 1973. Methionine-sulfoximine-resistant mutants of tobacco. Science 180: 1366-1368.

Carlson, P. S., H. H. Smith, and R. D. Dearing. 1972. Parasexual interspecific plant hybridization. Proc. Nat. Acad. Sci. 69: 2292-2294.

Chaleff, R. S. and P. S. Carlson. 1974. Somatic cell genetics of higher plants. Ann. Rev. Genet. 8: 267-278.

Cocking, K C. 1972. Plant cell protoplasts-isolation and development. Ann. Rev. Plant Physiol. 23: 29-50.

Gautheret, R. J. 1959. La culture des tissues vegetaux. Masson and Cie, Paris. 829 p.

Gibbs, J. L. and D. K. Dougall. 1965. The growth of single cells from *Nicotiana tabacum* callus tissue in nutrient medium containing agar. Exp. Cell Res. 40: 85-95.

Gupta, N. and P. S. Carlson. 1972. Preferential growth of haploid plant cells *in vitro.* Nature New BioI. 239: 86.

Harvey, A. K 1967. Tissue culture of *Pinus monticola* on a chemically defined medium. Can. J. Bot. 45: 1783-1787.

Harvey, A. E. and J. L. Grasham. 1969. The relative susceptibility of needle- and stem-derived white pine tissue cultures to artificial inoculation with mycelium of *Cronartium ribicola.* Can. J. Bot. 47: 1789-1790.

Harvey, A. E. andJ. L. Grasham. 1971. Inoculation of a nonhost tissue culture with *Cronartium ribicola.* Can. J. Bot. 49: 881-882.

Hattemer, H. H. 1963. Estimates of heritability published in forest tree breeding research, paper 2a/3. *In* Proc. World Consultation For. Genet. and Tree Improvement. Stockholm, Sweden.

Helgeson, J. P., J. D. Kemp, G. T. Haberlach, and D. P. Maxwell. 1972. A tissue culture system for studying disease resistance: the black shank disease in tobacco callus cultures. Phytopathology 62: 1439-1443.

Ingram, D. S. 1967. The expression of R-gene resistance to *Phytophthora infestans* in tissue cultures of *Solanum tuberosum.* J. Gen. Microbiol. 49: 99-108.

Ingram, D. S. 1973. Growth of plant parasites in tissue culture, p. *392-421. In* H. E. Street (ed.), Plant tissue and cell culture. Univ. of California Press, Berkeley, California.

Keller, W. A. and G. Melchers. 1973. The effect of high pH and calcium on tobacco leaf protoplast fusion. Z. Naturforschung. 28: 737-742.

Keller, W. A., B. L. Harvey, K. N. Kao, R. A. Miller, and O. L. Gamborg. 1973. Determination of the frequency of interspecific protoplast fusion by differential staining. Colloq. Intern. C.N.R.S. 212: 455-463.

Kimber, G. and R. Riley. 1963. Haploid angiosperms. Bot. Rev. 29: 480-531.

Kopecky, V. F. 1960. The experimental production of haploid white poplars *(Populus alba* L.). Silvae Genet. 9: 102-105.

Libby, W. J., R. F. Stettler, and F. W. Seitz. 1969. Forest genetics and forest-tree breeding. Ann. Rev. Genet. 3: 469-494.

Linsmaier, E. M. and F. Skoog. 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant. 18: 100-127.

Littlefield, J. W. 1964. Selection of hybrids from fibroblasts *in vitro* and their presumed recombinants. Science 145: 709-710.

Mathes, M. C. 1964. The culture ofisolated triploid aspen tissue. For. Sci. 10: 35-38.

Mathes, M. C. 1964. The *in vitro* formation of plantlets from isolated aspen tissue. Phyton 21: 137-144.

Nagata, T. and l. Takebe. 1971. Plating ofisolated tobacco mesophyll protoplasts on agar medium. Planta 99: 12-20.

Nakata, K. and M. Tanaka. 1968. Differentiation of embryoids from developing germ cells of tobacco. Jap. J. Genet. 43: 65-71.

Ogutuga, D. B. A. and D. H. Northcote. 1970. Caffeine formation in tea callus tissue. J. Exp. Bot. 21: 258-273.

Pelet, F., A. C. Hildebrandt, A. J. Riker, and F. Skoog. 1960. Growth *in vitro* of tissues isolated from normal stems and insect galls. Am. J. Bot. 47: 186-195.

Power, J. B., S. E. Commins, and E. C. Cocking. 1970. Fusion of isolated plant protoplasts. Nature 225: 1016-1018.

Reinert, J. and P. R. White. 1956. The cultivation *in vitro* of tumor tissues and normal tissues of *Picea glauca.* PhysioI. Plant. 9: 177-189.

Skoog, F. and C. O. Miller. 1957. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. Symp. Soc. Expt. BioI. 11: 118-131.

Staritsky, G. 1970. Embryoid formation in callus tissues of coffee. Acta Bot. Neerl. 19: 509-514.

Steward, F. C., A. E. Kent, and M. O. Mapes. 1966. The culture of free plant cells and its significance for embryology and morpho-genesis. Curr. Topics Develop. BioI. 1: 113-154.

Steward, F. C., M. O. Mapes, and P. V. Ammirato. 1969. Growth and morphogenesis in tissue and free cell cultures, p. 329-376. *In* F. C. Steward (ed.), Plant physiology, a treatise. Academic Press, New York.

Street, H. E. (ed.). 1973. Plant tissue and cell culture. Bot. Monogr., Vol. 11, Univ. of California Press, Berkeley, California. 502 p.

Sunderland, N. 1971. Anther culture: a progress report. Sci. Prog. (Oxford) 59: 527-549.

Tanaka, M. and K. Nakata. 1969. Tobacco plants obtained by anther culture and the experiment to get diploid seeds from haploids. Jap. J. Genet. 44: 47-54.

Tulecke, W. 1957. The pollen of *Gingko biloba: in vitro* culture and tissue formation. Am. J. Bot. 44: 602-608.

Vasil, V. and A. C. Hildebrandt. 1965. Differentiation of tobacco plants from single, isolated cells in microculture. Science 150: 889-892.

Vig, B. K. and E. F. Paddock. 1968. Alteration by mitomycin C of spot frequencies in soybean leaves. J. Hered. 59: 225-229.

Winton, L. L. 1968. Plantlets from aspen tissue cultures. Science 160: 1234-1235.

Winton, L. L. 1970. Shoot and tree production from aspen tissue cultures. Am. J. Bot. 57: 904-909.

Winton, L. L. 1971. Tissue culture propagation of European aspen. For. Sci. 17: 348-350.

Winton, L. L. 1972. Callus and cell cultures of Douglas-fir. For. Sci. 18: 151-154.

Winton, L. L. and D. W. Einspahr. 1968. The *use* of heat-treated pollen for aspen haploid production. For. Sci. 14: 406–407.

Withers, L. A. 1973. Plant protoplast fusion: methods and mechanisms. Colloq. Intern. C.N.R.S. 212: 517-545.

Wolter, K. E. 1968. Root and shoot initiation in aspen callus cultures. Nature 219: 509-510.

Wolter, K. E. and F. Skoog. 1966. Nutritional requirements of *Fraxinus* callus cultures. Am. J. Bot. 53: 263-269.

# PRECOCIOUS FLOWERING IN CONIFERS: THE ROLE OF PLANT HORMONES

Richard P. Pharis
Department of Biology,
University of Calgary,
Calgary, Alberta, Canada

Each year man must grow more wood on less land. While inroads into productive forest land from environmental degradation and industrial and urban uses may decline in the future, losses to agriculture, recreation, and special uses *(i.e.* wildlife and watershed protection) will continue. While the latter competing uses generally utilize forested land of marginal productivity, agricultural encroachment usurps the best timberland.

Thus, means by which the yield may be increased on the lands remaining for wood and fiber production are of great interest to the forest manager. Cultural techniques ranging from thinning, pruning and fertilization, to protection against disease and pests offer one approach, albeit an increasingly expensive one as labor and fossil fuel costs increase rapidly. A second approach is to reforest land with genetically superior trees, trees which may be produced from controlled genetic crossings in order to improve growth rate, fiber characteristics, resistance to insects or disease, or some other "plus" quality. The success of this second approach is dependent upon the ability of the geneticist to select and cross "plus" trees, and of the seed orchard manager to produce regular crops of abundant seed of the genetically superior progeny.

Problems relating to selection and testing of progeny may well be near solution, but unfortunately, the genetic crossing and seed production programs are significantly retarded by the inherent inability of most economically important conifers to flower until they are 10, 15, or even 20 years old (Giertych 1967, Puritch 1972). Maturation with significant seed set may not occur for another 20 years (see review by Puritch 1972). The period during which the tree is unable to flower is termed the "juvenile phase", and for herbaceous angiosperms is strictly defined as: "that phase in ontogeny during which seedlings cannot be induced to form flowers by any means" (Dooren-

bos 1965). However, this strict definition may be inappropriate for conifers because factors controlling their flowering are still poorly understood (Doorenbos 1965) and there are examples of extremely precocious spontaneous flowering (Smith and Konar 1969) as well as hormonally-induced flowering at ages of two to three months for species which do not normally flower for many years (Pharis *et af.* 1965; see also Figure 2). Though the reproductive structures of conifers are not flowers as defined for angiosperms, the term "flowering" will be used in this paper for sake of brevity to mean both the production of flowers in angiosperms and the production of mega- and micro-sporangiate strobili in gymnosperms.

The need of the geneticist for precocious flowering, and of the seed orchardist for not only precocious flowering, but also for continuous and regular production of seed, may be antithetical to successful selection and propagation of rapidly growing trees that will exhibit strong apical dominance through a long juvenile phase. Trees that can be crossed at an early age, and whose progeny produce abundant seed in the orchard, may be trees which divert significant amounts of photosynthate from wood production to pollen and ovulate cone crops (see review by Puritch 1972, Eis *et af.* 1965, Fielding 1960, Romberger 1967), and these latter characteristics are also heritable. Thus, the tree breeder and seed orchardist require a tool by which genotypes that normally flower sparsely, or not at all, can be induced to flower at will when necessary.

## Terminating the Juvenile Phase by Cultural Techniques

In nature the juvenile phase is terminated "as the tree slowly proceeds toward a state of ripeness-to-flower" (Klebs cited in Lang 1965). Howe"er, cultural treatments such as girdling, strangulation, root pruning, branch pruning, allowing the plant to become "pot bound", water stress, nitrogen stress and even fertilization (especially with nitrate), geotropic stimulation, changes in amount of radiant energy, and hormone treatment have all been used to increase cone production or induce precocious flowering. Many of the earlier experiments have been reviewed by Wareing (1959), Longman (1960), Doorenbos (1965), Robinson and Wareing (1969), Jackson and Sweet (1972), Puritch (1972) and Pharis and Kuo (1975), and will be discussed only briefly in the present paper.

Several workers *(e.g.* Doorenbos 1965, Robinson and Wareing 1969) have urged that a clear distinction be drawn between treatments whch induce the seedling to reach the "ripeness-to-flower" state and treatments which promote flowering in an already adult plant. However, a perusal of the original literature for the treatments mentioned above indicates that many treatments which "promote" flowering in adult, but otherwise nonflowering trees, will also cause, on occasion, precocious flowering in juvenile-phase seedlings. Thus, I am not so certain that such a "clear distinction" does exist.

It has been emphasized by Owens (1969) that in certain conifers *(e.g.* Douglas-fir, *Pseudotsuga menziesii* [Mirb.] Franco) cultural treatments which enhance flowering may only do so by influencing differentiation and/or development rather than strobilus initiation, since the potentially reproductive (but undifferentiated) primordia are initiated well before treatments have begun. In other conifers, such as Arizona cypress *(Cupressus arizonica* Greene) and western redcedar *(Thuja plicata* Donn.), strobili are initiated directly from previously vegetative meristems (Owens and Pharis 1967, 1971). Thus, in order to properly time application of an inductive treatment one should first determine into which pattern of primordia initiation, differentiation, and development the conifer in question fits.

Most, but not all, of the treatments referred to above result in elevated levels of carbohydrate in the differentiating bud and adjacent stem and foliar tissue, often resulting in an elevated carbon nitrogen (C/N) ratio. While much of the past work indeed shows a high C/N ratio in flowering tissue, the causal nature of the relationship remains obscure. Carbohydrate levels might be a limiting factor for strobilus differentiation and early development, and high carbohydrate levels may be necessary for sexual differentiation and development, or they may trigger some hormonal mechanism which in turn promotes flowering. Or, carbohydrates may play no direct role and high concentrations may merely be the natural consequence of diminished utilization in vegetative growth. Reduced levels of nitrogen will elevate the C/N ratio and induce precocious flowering in cryptomeria *(Cryptomeria japonica* D. Don; Lyr and Hoffmann 1964) and Arizona cypress (Kuo 1973). However, nitrogen fertilization, especially in the nitrate form, will also promote both increased flowering and early flowering in many members of the Pinaceae (see reviews by Puritch 1972, Ebell 1972a, b, Ebell and McMullan 1970, Schmidtling 1971). These conflicting results cast

doubt on any general hypothesis that a high C/N ratio is *de rigeur* for flowering.

### *Gibberellin Application, the Most Successful of the Cultural Techniques*

Of all the methods by which precocious flowering can be induced or flowering promoted in adult phase conifers, the exogenous application of the plant hormone gibberellin (GA) stands out as the most successful and readily repeatable procedure. Kato, *et al.* in 1959 first demonstrated that strobili could be induced in juvenile phase seedlings of cryptomeria and this early work was extended to a large number of conifer species within the Cupressaceae and Taxodiaceae by numerous workers (Hashizume 1959, 1966, 1973, Kato *et al.* 1958, 1959, Pharis *et al.* 1965, Pharis and Morf 1967, 1968, 1969). Most experiments have been accomplished using $GA_3$ (Figure 1). With only one exception (Hashizume 1967), significant flowering using GAs was restricted to conifers within the Cupressaceae and Taxodiaceae until very recently (Ross and Pharis 1973, Pharis *et al.* 1974, Wample and Pharis 1975). The results are often quite spectacular, as shown by precocious flowering in very young seedlings only 3 to 4 cm high (Figure 2), and massive production of strobili in older plants (Figure 3). Induced strobili are readily visible under the light microscope by day 20 from beginning of treatment (Figure 4), and to the naked eye by day 30 in Arizona cypress.

### *Factors Affecting Gibberellin-Induced Flowering in the Cupressaceae and Taxodiaceae*

In evaluating ways in which success in promoting flowering in the Cupressaceae and Taxodiaceae with GAs could be repeated using more economically important members of the Pinaceae, two avenues of approach are apparent. One involves learning more about the role(s) of GAs in the induction, differentiation, early development, and maturation of strobili for species within the Cupressaceae and Taxodiaceae, and as well, finding out what else (*i.e.* environmental, nutritional, and other hormonal factors) affect these GA-mediated processes. The second approach involves correlative studies of endogenous hormone levels in the Pinaceae under circumstances where flowering can be induced or promoted by cultural means.
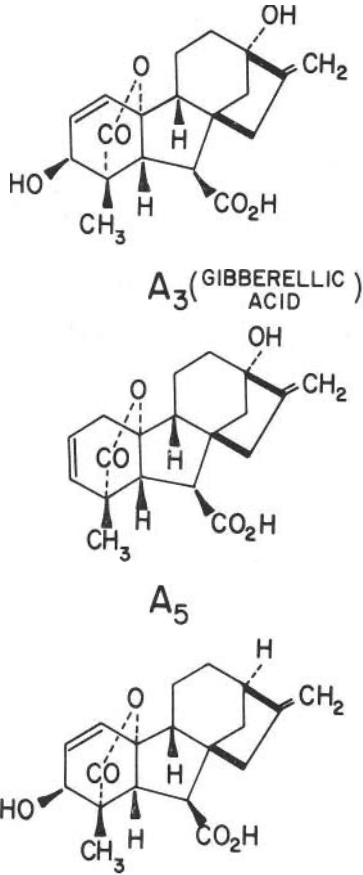
# STRUCTURES OF GIBBERELLINS



Figure 1. Structure of several gibberellins known to be effective in inducing or promoting /lowering in conifers.

Suspect hormones can then be tested in field trials. The first approach is discussed below in some detail. A brief report of the preliminary research that we have done using the second approach follows.

Figure 2. Western redcedar seedling, age 4 months. Initiation of strobili was induced by spraying with gibberellin $A_3$ at age 3 months. The male strobilus is developing at the top of the plant. (From Pharis and Owens 1966).

Within a few years of the initial discovery that $GA_3$ would readily induce precocious flowering in some conifers, research into various aspects of flowering in the Cupressaceae and Taxodiaceae was initiated by Hashizume, Kato, and Migita in Japan, Lyr and Hoffmann in

Figure 3. Arizona cypress seedling, age 16 months, sprayed with $GA_3$ for 4 months. There are 8,009 meristems on this plant, most of which were induced to become reproductive by gibberellin. Only about 50 of these strobili are female. (From Pharis and Owens 1966).

the DDR, and Glenn, Kuo, Morf and myself at the University of Calgary in Canada.

*Methods of Hormone Application.* Many workers have used lano-
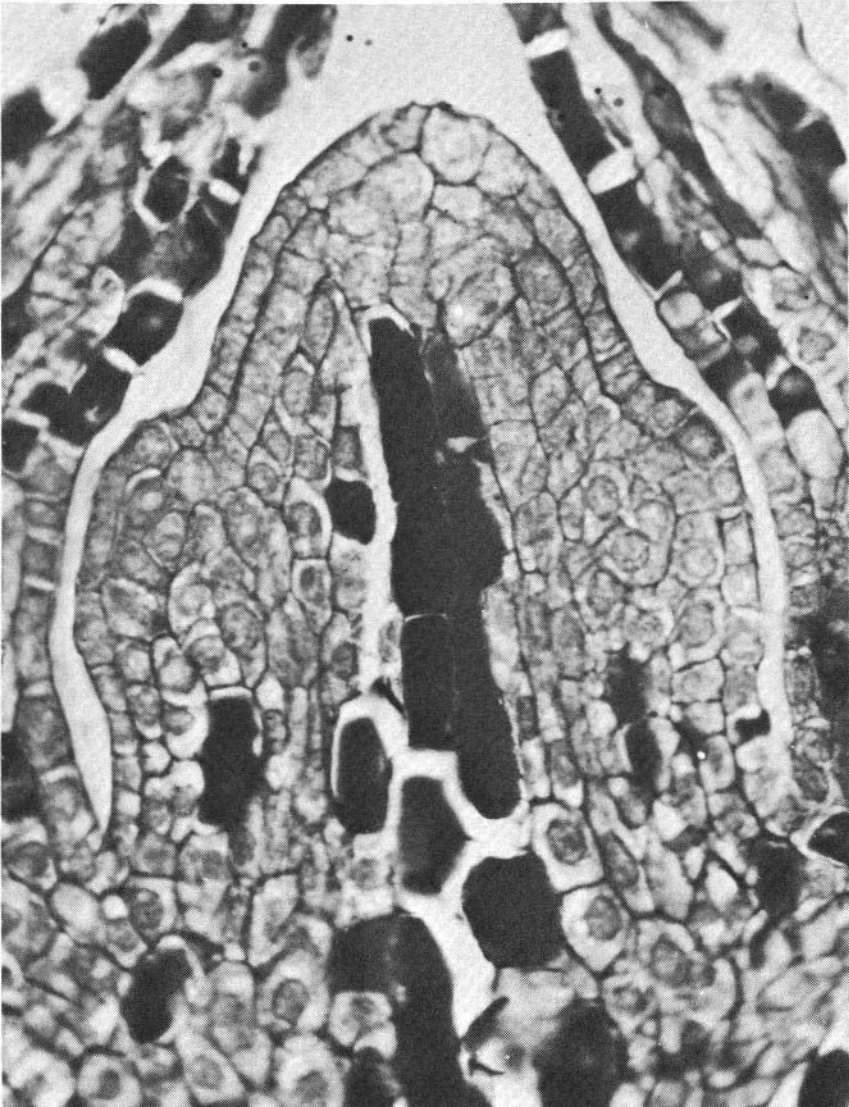
Figure 4. A. Longitudinal section through the apex of a branch on an Arizona cypress seedling used as a control in a gibberellin experiment. It received only a spray of water and detergent and retains the form of a typical vegetative apex (450 x).

lin paste or aqueous sprays ranging from 100 to 500 mg!1 of *GA₃*, usually with a surfactant. We have looked at other methods of application, and Glenn (1973), working in our lab, found that for experi-
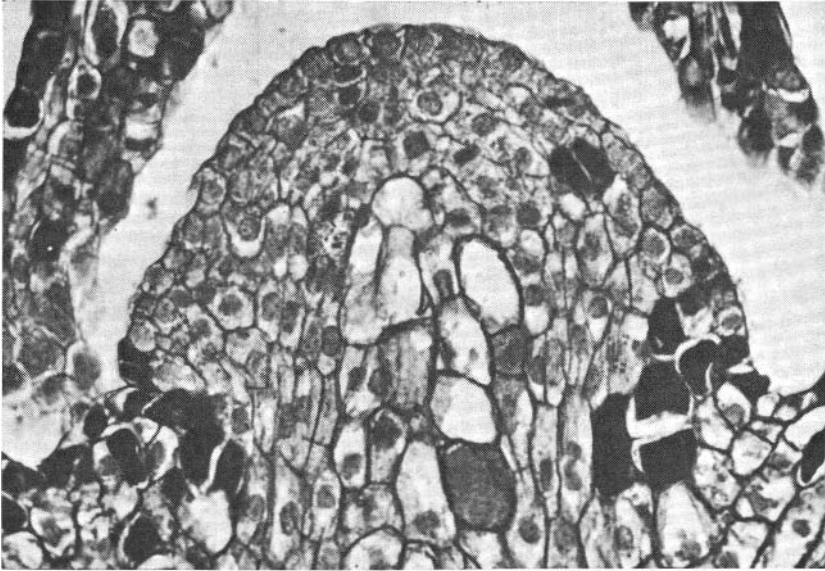
Figure 4.  B. As above, except that the cypress was sprayed with $GA_3$. The apex, fixed 20 days after initial treatment with gibberellin, has the characteristic enlarged form of a reproductive apex (450 x). (From Pharis and Owens 1966).

mental purposes a soil drench of about 100 ml of 100 or 500 mg/1 of $GA_3$ produced a very uniform and high level of flowering. A single application of the 500 mgt 1 dosage, left in contact with the roots for 24 hours and then flushed with distilled water, was more effective than single applications of 100 mgt 1, or repeated applications of 100 or 500 mgt 1 of $GA_3$. Whereas foliar applications at high (500 mg/1 or more) concentrations, and under high light intensity (3,000 ft-c or more), caused toxicity, the problem was eliminated with the single root drench at 100 mg/I. Another satisfactory technique has been the injection of 1 to 400 $\mu g$ of GAs in 1 to 5 $\mu l$ of 80 to 95% EtOH into the phloem and xylem tissue of the woody branch or main stem (Kuo 1973, Pharis and Morf 1972). Dose:response values of 0.05 $\mu g$ $GA_3$/strobilus have been noted (Pharis and Morf 1972). Injection produces some scarring, but damage is minimal and no toxicity has been noted. The technique is especially valuable for research using radioactive GAs. Movement from point of injection in the middle stem portion of a seedling of Arizona cypress is invariably upward (less than 15% moves into the lower shoot and root), and by day 10, over 50% of the applied radioactivity has been mobilized into the upper shoot (Kuo 1973, Pharis *et al. 1972).*

*Age of Plant and Amount of GA Applied.* Both Hashizume (1959, 1966, 1967) and Kato *et al.* (1959) using cryptomeria noted that the effect of $GA_3$ on strobilus initiation was greater on older trees, and the effect increased with dosage. In young seedlings, elevated $GA_3$ concentrations enhanced production of ovulate strobili while lower $GA_3$ concentrations produced only staminate strobili. A similar situation was noted for western redcedar re sexuality (Pharis and Morf 1968, 1970) and for dawn redwood *(Metasequoia glyptostroboides* Hu et Cheng) and Hinoki cypress *(Chamaecyparis obtusa* Sieb. et Zucc.) re the age effect and sexuality (Hashizume 1973). Using Arizona cypress we have noted that very young plants require much more exogenous $GA_3$ than older ones to give the same flowering response, and that $GA_3$ concentrations which produce profuse flowering in year-old plants produce no strobili on 2 to 4 month-old plants (Pharis *et al.* 1965). Thus, we have suggested that the juvenile phase in Arizona cypress may be a period of time during which endogenous GAs have not yet attained sufficient concentration to cause flower initiation (Pharis and Morf 1968). During these early years, endogenous GAs might be utilized preferentially for vegetative growth, whereas, as the seedling grows, external conditions *(i.e.* competition for soil water and nutrients, or traumas such as insect or disease attack) may diminish vegetative growth, thus allowing a GA build-up sufficient to initiate strobilus formation.

*Light Intensity.* Puritch (1972) reviewed a number of anecdotal reports concerning light intensity and flowering, the majority of which concluded that light intensity was responsible for the increased flowering response noted in free-growing trees, in trees growing on south slopes, or on that side of the tree facing the sun. However, these reports do not differentiate between light intensity *per se* and the increase in temperature which usually accompanies increased light intensity. The location of ovulate strobili on the upper and outside branches, as opposed to staminate strobili which are located on the inner portions of branches and in the lower crown has been attributed to differences in light (see review by Puritch 1972), although a more direct effector may be hormone concentration, perhaps regulated via light and temperature-dependent photosynthate production.

Glenn (1973) found that flowering in GA-treated Arizona cypress grown under 1,000 or 8,000 $\mu w/cm^2$ was highly dependent upon light intensity (Figure 5). In these experiments, air temperature was
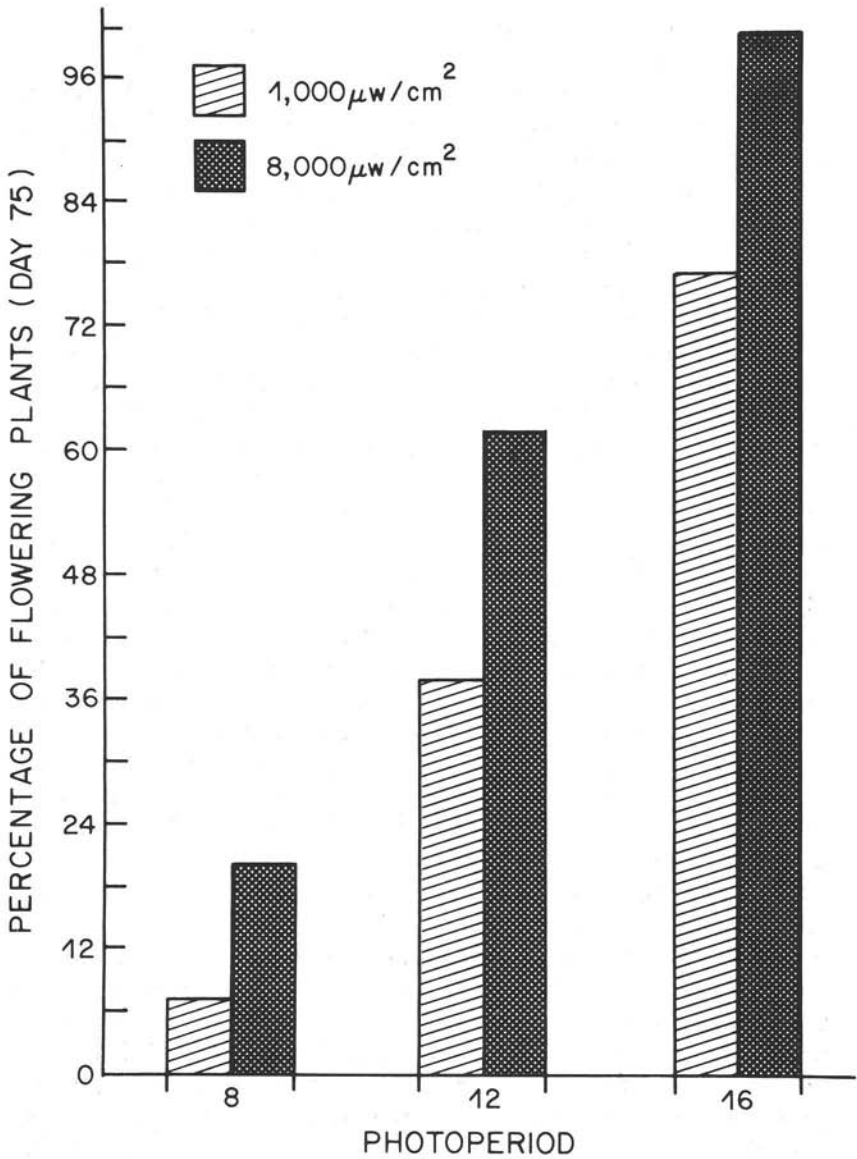
Figure 5. Effect of radiation flux density on the inductive response of Arizona cypress seedlings to an aqueous spray of 100 mg/1 of $GA_3$ under 8, 12 and 16 hour photoperiods. (Courtesy of J. L. Glenn 1973).

maintained at a constant value through the use of controlled environment chambers.

It is logical to assume that the main effect of increased light intensity would be the provision of adequate substrate for strobilus development, but a direct effect on endogenous hormone (including GAs) concentration should not be ruled out (see discussion later on photoperiodic effects and endogenous GA concentration).

*Photoperiod and Low Temperature.* Photoperiodism as a control mechanism for regulating flowering response in herbaceous plants was first noted in 1920 by Garner and Allard. The literature on photoperiodic control of flowering has been reviewed extensively, most recently by Evans (1971) and Hillman (1969). Photoperiodism can be defined as: "a response to the time of light and darkness such that total light energy and intensity above a relatively low level are secondary to duration and succession of light and dark periods" (Hillman 1969). With regard to flowering, there are three basic types of photoperiod responses: plants which are neutral in the response to daylength (DNP); plants responsive to short daylength (SDP); and plants responsive to long daylength (LDP). Modifications or combinations of these basic types also exist. The ability of a plant to sense photoperiodic differences is thought to reside in a photoreceptor pigment (phytochrome) which exists in two photoconvertible forms (see review by Kroes 1970). An oft used test to determine the extent of a plant's dependence upon photoperiodism to regulate a physiological process or developmental sequence is to interrupt a long dark period with a brief light break. This would be unfavorable for flowering in SDP and favorable in LDP.

The extended juvenile period of most conifers, coupled with a size inconvenient for controlled environment facilities, has hampered research on photoperiodism in trees with regard to flowering. It is not clear whether strobilus initiation, or early development, or both are under photoperiodic control in conifers. Nor, is it easy to separate the effects of light intensity (or even the total number of hours of photosynthetically useable light) from that of photoperiod. Further complicating the issue is the fact that staminate strobili appear to have different photoperiod requirements (LD) from ovulate strobili (SD), although recent research indicates that in nature this may be more a factor of sequential "increases" or "decreases" in daylength rather than length of the day *per se* (Owens and Pharis 1971). Not only may initiation and early development of the strobilus be under
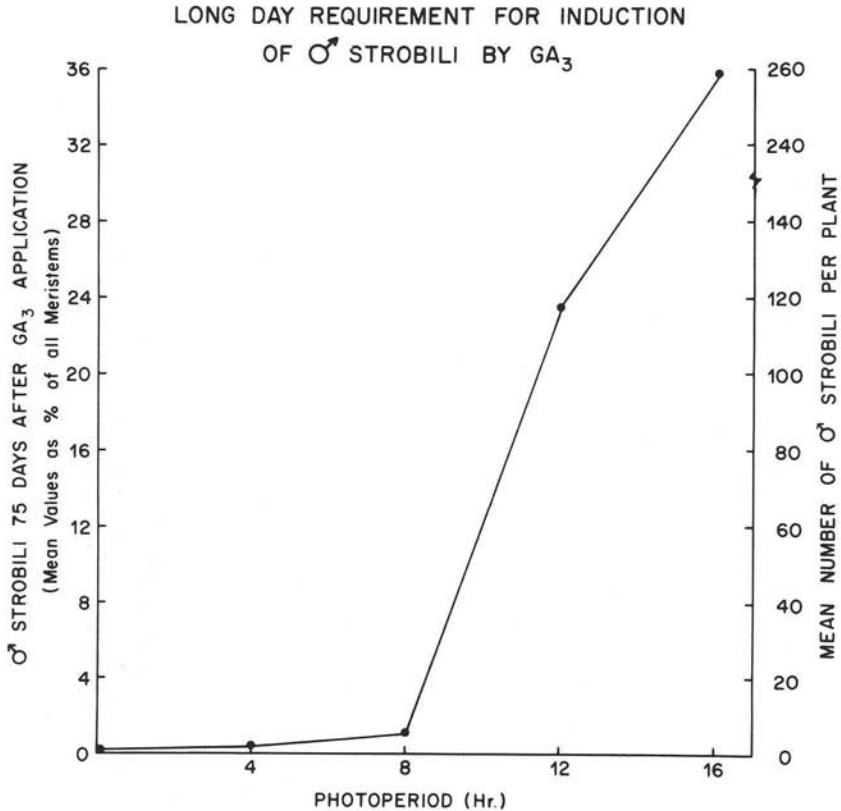
Figure 6. Effect of photoperiod on induction of staminate strobili by 100 mg/1 $GA_3$ aqueous spray on 6- to 8-month-old Arizona cypress seedlings kept in darkness and by weekly application of 100 ml of aqueous $GA_3$ solutions as a soil drench in other photoperiods. Plants in 4- and 8-h photoperiods received 500 mg/1 $GA_3$, while those in 12- and 16-h photoperiods received 100 mg/1 solutions. It should be noted that even $GA_3$ applications up to 2,000 mg/1 in aqueous spray would not induce strobili in total darkness. (Courtesy of J. L. Glenn 1973).

photoperiodic control, but subsequent development to anthesis (and beyond?) may also have a photoperiodic component.

The role of daylength (but not necessarily photoperiod) has been implicated in controlling the proportion of ovulate and staminate strobili in pines (Giertych 1967, Goo 1968), cryptomeria (Hashizume 1959, 1966, 1973), and western redcedar (Pharis and Morf 1968, 1970), and in the initiation and subsequent early development of strobili in Arizona cypress and western redcedar (Pharis and Morf

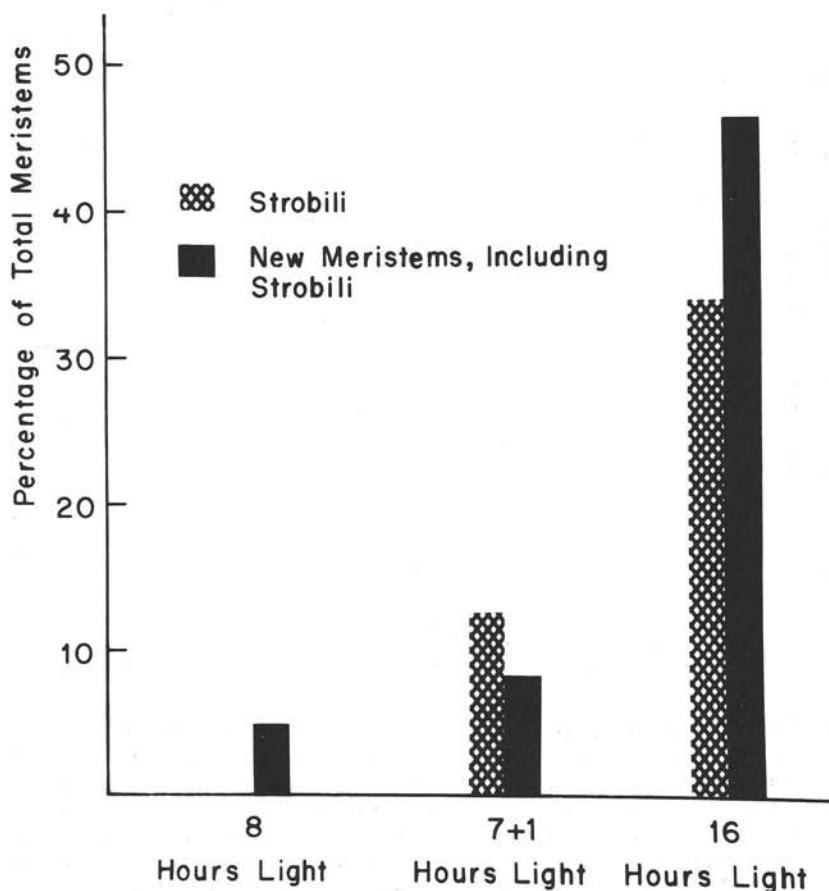DAY 50 AFTER INITIATION OF
500 ppm $GA_3$ SPRAY



Figure 7. Effect of photoperiod on production of new meristems (vegetative plus reproductive) and staminate strobili under 8 h of light (continuous, or with 1 h given in middle of dark period) or 16 h of light. Spray with 500 mg/1 $GA_3$ began 14 days after transfer of 7-month-old plants from LD to SD. (From Pharis *et al.* 1970).

1967, 1968, Pharis *et al.* 1970, Glenn 1973; see Figures 6 and 7). That the requirement for LD in the initiation of staminate strobili induced by $GA_3$ is at least in part photoperiodic in nature is shown by experi-
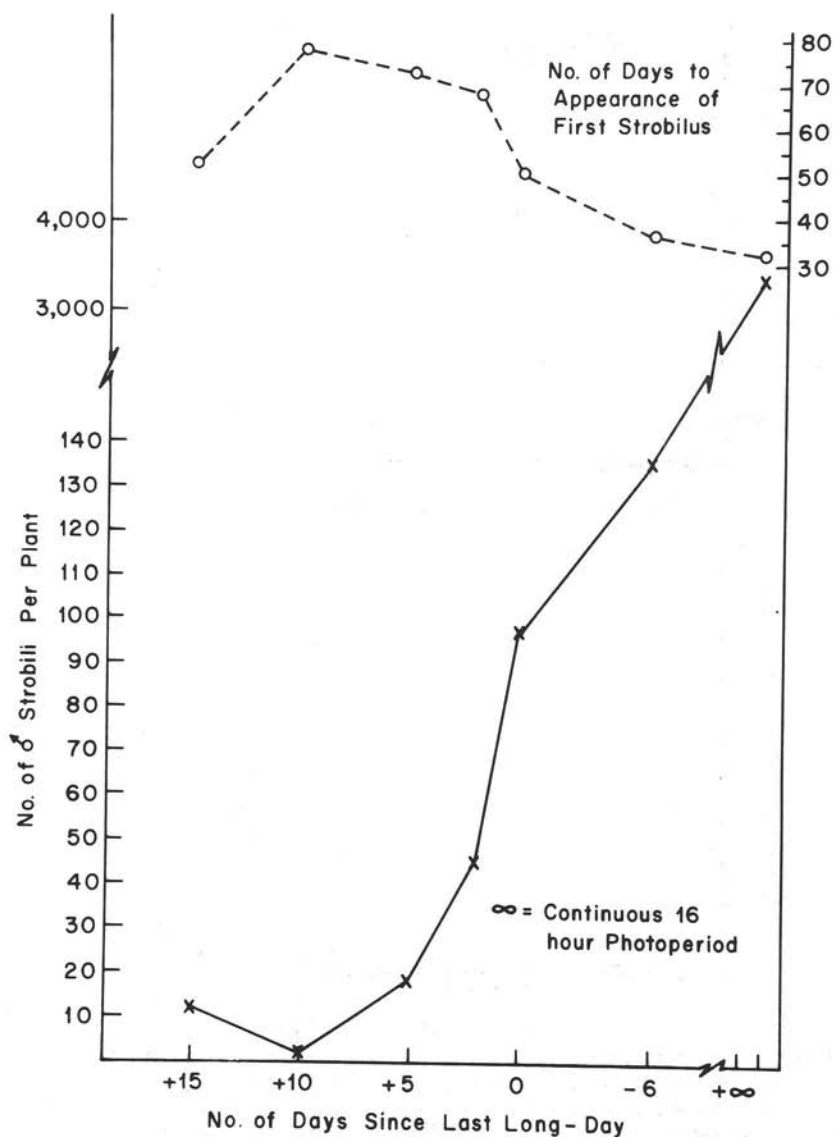
Figure 8. Lower curve: number of staminate strobili induced in Arizona cypress under short-day (SD) with $GA_3$ administered by aqueous spray over a 6-month period beginning 15, 10, 5, 2, or 0 days since last long-day (LD) or 6 days before first SD. Plants kept continuously under LD shown on far right ($\infty$). Upper curve: days to the appearance of the first strobilus under the same conditions. (From Pharis *et al.* 1970).
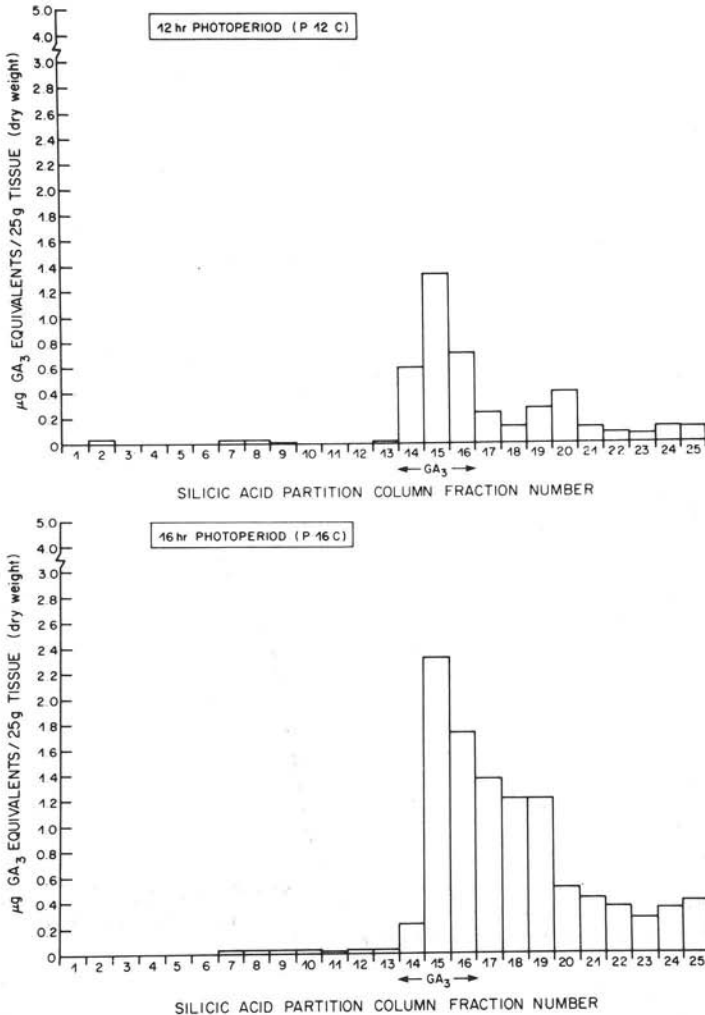
Figure 9. Effect of photoperiod on levels of endogenous GA-like substances in Arizona cypress seedlings. Tissues were extracted with 80% methanol, and the acidic, ethyl acetate-soluble fraction purified by use of insoluble polyvinylpyrrolidone and charcoal:celite prior to chromatography on silica gel partition columns. Each fraction was tested by serial dilution on the dwarf rice *cv.* Tan-ginbozu "microdrop" bioassay. (Courtesy of J. L. Glenn 1973).

ments on Arizona cypress where the long night has been interrupted by a light break with white light (Figure 7; Glenn 1973, Pharis *et al.*
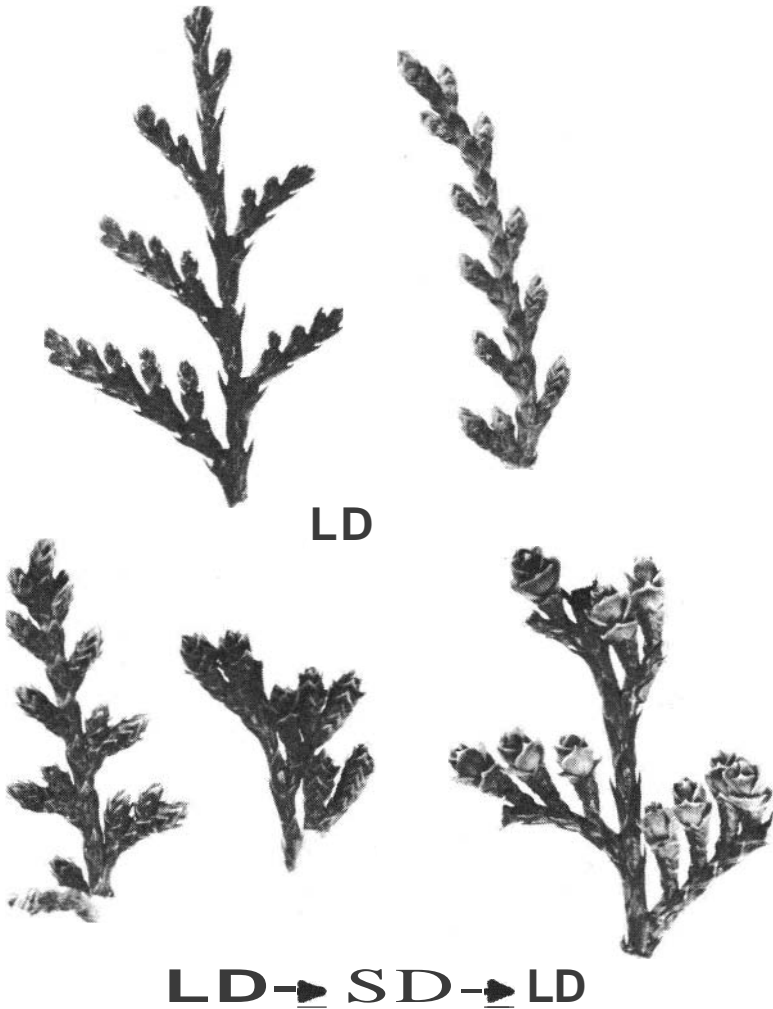
**LD**

**LD→ SD→LD**

Figure 10. Development of ovulate strobili in western redcedar in response to LD - SD - LD photoperiodic sequence. Branches from plants kept under LD (upper half) show normal vegetative state (left) of untreated plant, and a state of arrested development for ovulate strobili sprayed with 100 ppm $GA_3$ for 7 months (right). Strobili stay in this state of arrested development for at least as long as 20 months if kept under LD. Branches from plants sprayed with 100 ppm $GA_3$ for 7 months, but given 4 months of LD, 1 month of SD, and 2 months of LD (lower half) during the 7 months show full development. Approximately 3 weeks after end of SD, bracts loosen (left), expansion begins (middle), and within 2 to 3 weeks development of the strobilus is partially completed (right). (From Pharis and Morf 1968).
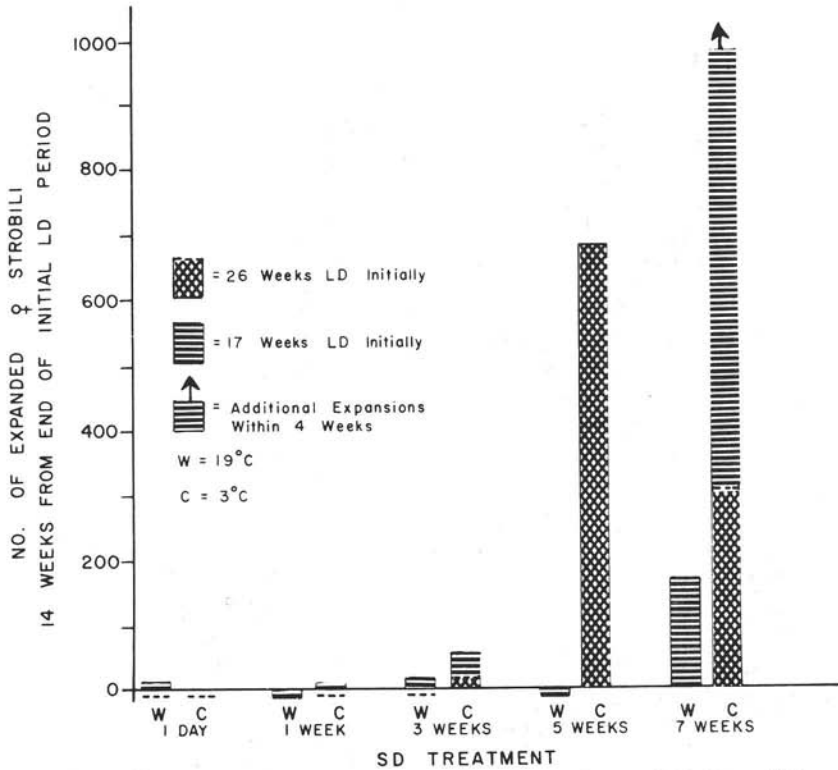
Figure 11. Effects of short-day (SD) under warm (19° to 22°C) or cold (3°C) conditions on the continued development of the ovulate strobilus. One set of plants was initially given $GA_3$ over 17 weeks of long-day (LD) at 22°C, prior to being subdivided into 9 groups and given 1 day, 1, 3 or 7 weeks of SD (3° and 19°), or 5 weeks of SD at 19°C. A second set of plants was given $GA_3$ over 26 weeks of LD at 22°C, then subdivided into 3 groups and given 3, 5 or 7 weeks of SD at 3°C. Expanded strobili were expressed as total number per four plants. Additional strobilus expansion did not occur after week 14 (from end of initial LD) except for plants receiving 7 weeks of cold SD after an initial LD period of 17 weeks. (From Pharis *et al.* 1969).

1970). If the night break is given as red light (R), flowering is still promoted, but following R with far-red light (FR) enhances rather than reverses the R effect. A positive effect for FR in flowering of a number of other plants was noted by Evans (1971).

Arizona cypress seedlings also retain a "memory" of their previous photoperiodic environment when transferred from LD to SD (Fig-
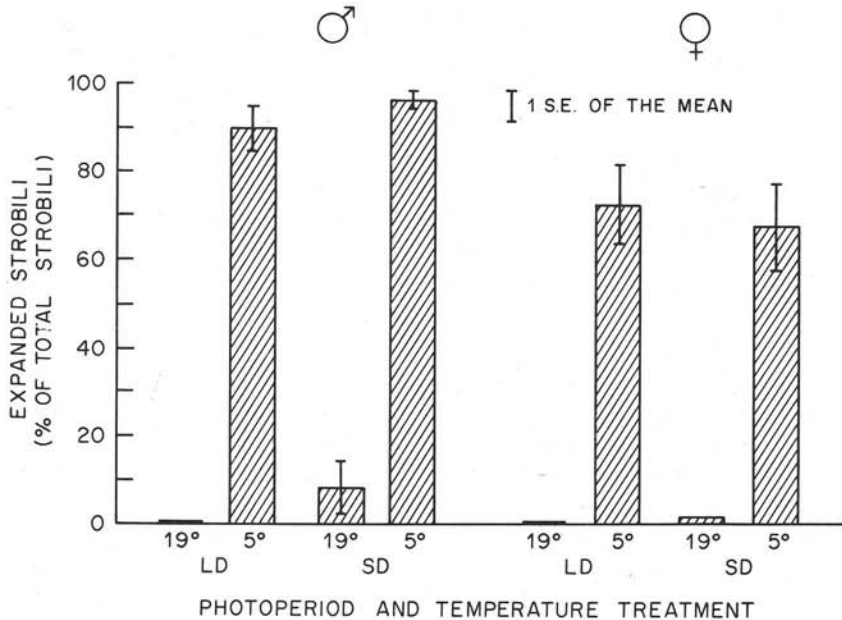
68

Figure 12. Effect of photoperiod and temperature on the expansion of ovulate (♀) and staminate (♂) strobili of western redcedar. During 21 weeks of long day (LD) at 19°C, strobili were induced by exogenous application of GA₃. Then 8 weeks of treatment (*i.e.* LD and short-day (SD) at either 5° or 19°C) was given, followed by 6 weeks of LD at 19°C. (From Pharis and Morf 1972).

ure 8; Glenn 1973, Pharis *et al.* 1970). It is possible that this memory may be residual amounts of endogenous GA produced under LD, because in Arizona cypress increasing daylength (*i.e.* photosynthetically useable light) results in increased endogenous GA levels (Figure 9; Glenn 1973).

Daylength is also one of the factors influencing development of both staminate and ovulate strobili to the stage of anthesis (Pharis and Morf 1968, 1972, Pharis *et al.* 1969) as is shown by Figures 10 and 11, although low temperature appears to be the major factor and short days only a nominal factor (see Figure 11 and Pharis and Morf 1972).

The requirement for low temperature and/or short days for development of the strobilus to the stage of anthesis is not an unexpected adaptation for temperate zone trees since induced strobili normally overwinter prior to anthesis. Work several years ago at the University

of Calgary (Pharis and Morf 1968, unpublished data) indicated that one *Cupressus* species, Mexican cypress (G. *lusitanica* Mill. of Central America origin) had no low temperature requirement for expansion of strobili to the stage of anthesis, Arizona cypress (southern California origin) and Mendocino cypress (G. *pygmaeae* [Lemm.] Sarg. of northern California origin) had a nominal cold requirement. However, western redcedar (British Columbia origin) had a lengthy cold requirement (Figures 11 and 12 and Pharis and Morf 1972). Long periods of time *(i.e.* 12 to 20 months) under warm, long days will result in a small percentage of the strobili proceeding to anthesis for the *Cupressus* species, but this treatment has no effect on western redcedar (Pharis *et al.* 1969, Pharis and Morf, unpublished data). Thus, evolution at northern latitudes may have resulted in a stricter control mechanism for this photoperiod/low temperature phenomenon. A requirement of this type implies that an endogenous timing mechanism be operative. Differential temperature optima for synthesis and/or degradation of inhibitory and promotive substances could result in different concentrations of these compounds, which could in turn maintain the state of arrested development, or trigger anthesis-like growth (see reviews by Vegis 1964 and Wareing and Saunders 1971 for analogous examples).

*Endogenous Levels of GA-like Substances.* As mentioned earlier, experiments in which age of seedlings and/or concentration of exogenous $GA_3$ were varied, suggested that the response to be expected from anyone level of exogenous $GA_3$ treatment might depend upon the endogenous concentration of GA-like substances. Certainly, in situations where the endogenous GA level can be increased *(i.e.* by lengthening the day, see Figure 9), a greater flowering response could be produced by a given amount of exogenous $GA_3$ (Glenn 1973).

Similarly, application of a growth retardant AMO-1618[1] known to inhibit GA biosynthesis in higher plants (see review by Lang 1970) and reduce the endogenous GA levels in Arizona cypress (Kuo 1973) will reduce the number of strobili induced by a given amount of exogenous $GA_3$. Thus, there is a negative correlation between the number of strobili produced by a given amount of $GA_3$ and the concentration of growth retardant applied. Conversely, a positive

---

[1] 14-hydroxyl-5-isopropyl-2-methylphenyl trimethyl ammonium chloride, I-piperidine carboxylate.

correlation between the flowering response induced by a given amount of exogenous $GA_3$ *and* the concentration of endogenous GA-like substances exists (Kuo 1973). If it is assumed that AMO-1618 has no direct effect on the action of applied $GA_3$, then this may also be taken as indirect evidence that exogenous $GA_3$ supplements endogenous GA levels.

When flowering was induced by low N nutrition in Arizona cypress (Kuo 1973), the response was correlated with an increase in endogenous GA levels, especially GA-like substances of a relatively non-polar nature. Similar correlations exist for members of the Pinaceae, and will be discussed below.

### Can Successes Comparable to Those in the Cupressaceae and Taxodiaceae Be Obtained With the Pinaceae?

The successful manipulation of flowering, especially the induction of precocious flowering, in commercially important species of the Pinaceae would be a practical extension of the past work on precocious flowering in the Cupressaceae and Taxodiaceae. Until very recently, success in this endeavour appeared highly unlikely. However, work with Japanese larch (*Larix leptolepis* [Sieb. et Zucc.] Gordon; Hashizume 1967), Douglas-fir (Ross and Pharis 1973, Pharis *et al.* 1974), lodgepole pine (*Pinus contorta* Dougl.; Pharis *et al.* 1974, Wample and Pharis 1975) and loblolly pines (*Pinus taeda* L.; Wample and Pharis 1975) has shown gibberellins to be quite successful in promoting flowering in these species of the Pinaceae. Hashizume (1967) used exogenous applications of $GA_3$ or NAA[2] on Japanese larch. Ross and Pharis (1973) found a $GA_{4/7}$ mixture best for non-flowering Douglas-fir grafts. Two-year-old seedlings of Douglas-fir responded best to a high dosage of the $GA_{4/7}$ mixture, and 100 percent flowering (more than 7 female strobili per branch) was obtained on 6-year-old seedlings with $GA_{4/7} + GA_9 +$ low levels of NAA (Pharis *et al.* 1974). Both lodgepole seedlings and loblolly non-flowering grafts responded best to treatments containing high levels of $GA_{4/7}$ (Wample and Pharis 1975). In certain of the above experiments, non-destructive girdling was used to enhance the treatment

---

[2]naphthaleneacetic acid
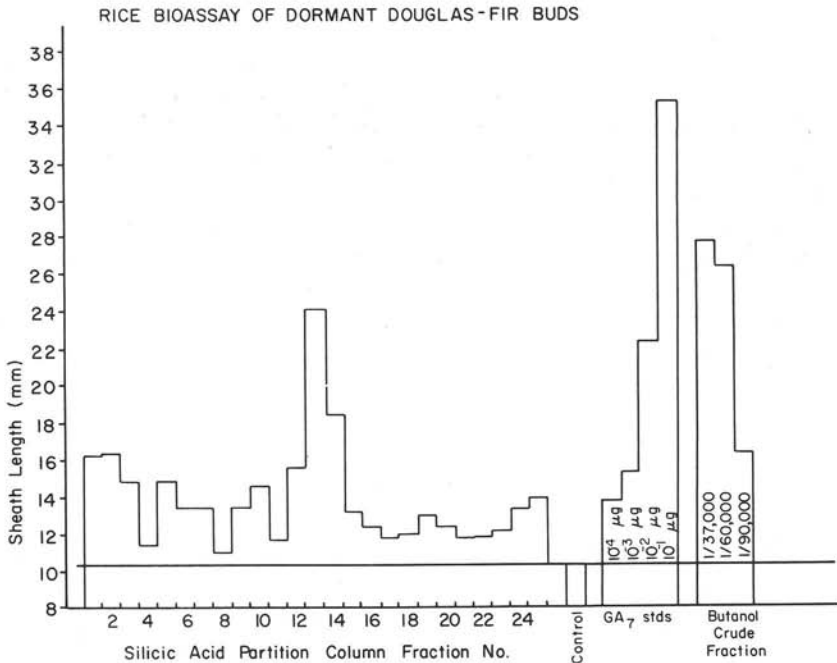
RICE BIOASSAY OF DORMANT DOUGLAS-FIR BUDS



Figure 13. Biological activity of GA-like substances from an extract of over 10,000 dormant buds of Douglas-fir collected in early March. Bioassay of acidic, ethyl acetate-soluble fraction (acidic free GAs) is on left. Each fraction from a silica gel partition column was assayed at 1/1000 aliquot on dwarf rice *cv* Tan-ginbozu. Elution pattern of standard GAs as per Figure 14. Activity of butanol soluble GA-like substances (highly polar acidic GAs or conjugated GAs) shown on far right at 3 dilutions (1/37,000, 1/60,000, and 1/90,000). (From Pharis and Rediske, unpublished data).

effect. Girdling alone has no significant effect on the promotion of flowering.

The successes with Douglas-fir and lodgepole and loblolly pine were achieved with the topical application of high concentrations (up to 400 $\mu$g/bud/application) of certain of the non-polar GAs (*i.e.* $GA_{4/7}$ mixture, $GA_5$, $GA_9$) during that period of the year when sexual differentiation would occur in the adult phase (Owens 1969). Guidance as to which GAs to use was provided by studies in which the endogenous GAs of conifers under situations known to promote flowering (*i.e.* drought, nitrate fertilization, girdling, low N nutrition) were analyzed and compared to control, non-flowering situations. In each case, flowering was correlated with an increased endogenous
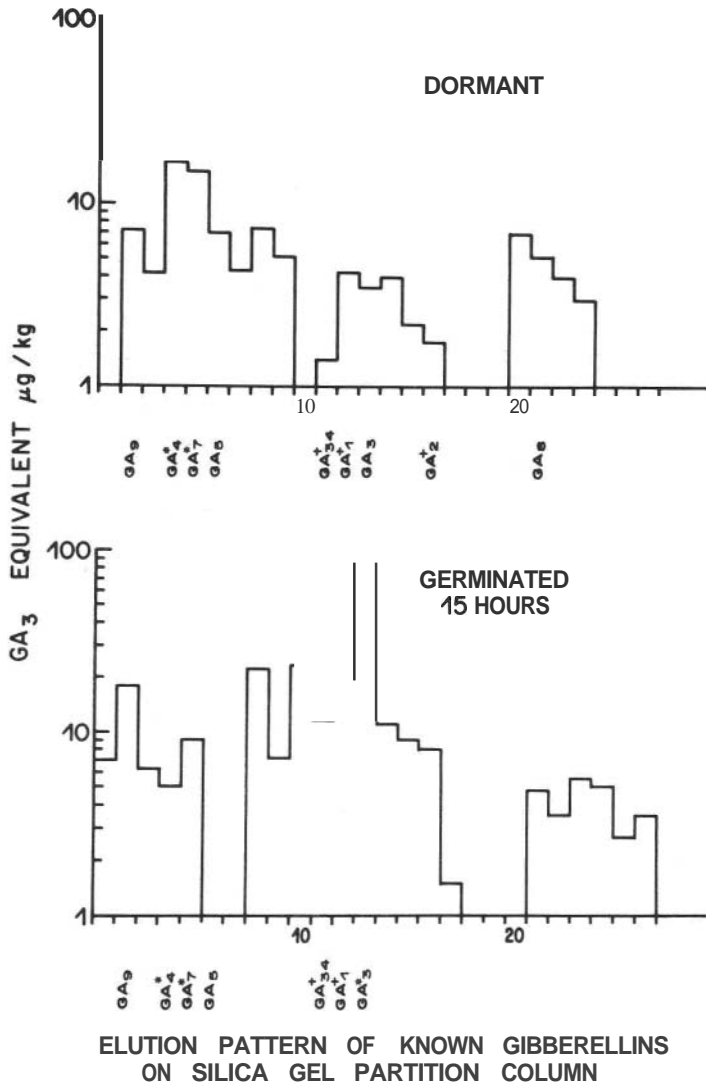
72

ELUTION PATTERN OF KNOWN GIBBERELLINS
ON SILICA GEL PARTITION COLUMN

Figure 14. Gibberellins and gibberellin-like substances in dormant and germinated pollen of knobcone pine *(Pinus attenuata* Lemm.). Concentration of endogenous GAs and GA-like substances expressed as **µg** per kg (dry weight) of pollen for each fraction of a silica gel partition column which was bioassayed in serial dilution on dwarf rice *cv* Tan·ginbozu. Symbols: * = native GA as determined by gas chromatography-mass spectrometry; + = native GA as deducted from the feeding of tritiated $GA_4$ (1, 2-[3H].$GA_4$) to the germinating pollen. (From Kamienska *et al* 1973).

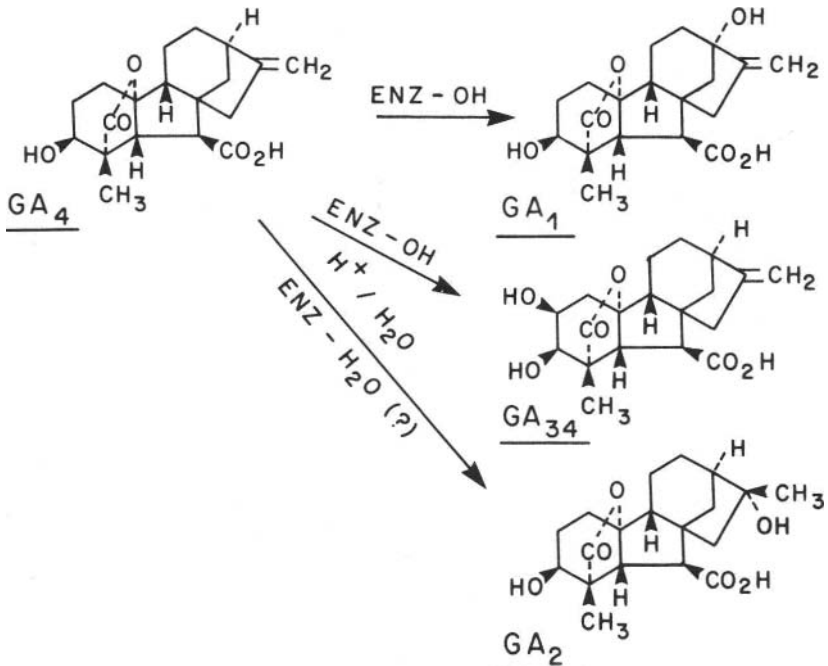## PROBABLE   MECHANISMS   OF   CONVERSION



Figure IS. Probable mechanism of conversion of 3H-gibberellin $A_4$, a native GA of germinating pollen of knobcone pine (from Kamienska, Durley, and Pharis, unpublished data). A similar mechanism is also probable for conversion of 3H-GA$_4$ to 3H-GA$_{34}$ and *3H-GA$_Z$* in needles of young Douglas-fir seedlings (Wample, Durley, and Pharis, unpublished data). 3H-GA$_i$ is not produced from 3H-GA$_4$ by Douglas-fir in detectable quantities. Gibberellin A$_4$ has not yet been characterized from Douglas-fir, but a mixture of GA$_{4/7}$ will promote /lowering in Douglas-fir grafts (Ross and Pharis 1973, 1975) and young seedlings (Pharis and Ross 1975).

level of relatively non-polar GA-like substances (Kuo 1973, Pharis, Ebell, and Rediske, unpublished data). Conversely, the vegetative state was correlated with a high level of endogenous GA$_3$ and other polar GA-like substances and reduced levels of the non-polar GA-like substances. The endogenous GAs of certain of the conifers were characterized by bioassay (Figure 13), and by chemical means (Figure 14; Kamienska *et al.* 1973). That high dosage levels *(i.e.* greater than 400 μg/shoot/application) are warranted is indicated by the rapidity with which conifers metabolize the relatively non-polar GAs

to other acidic, more polar GAs (see Figure 15) and more polar conjugated products (Kamienska *et al.* 1973, Wample, Durley, and Pharis unpublished data).

*Conclusions*

Manipulation of flowering in members of the Pinaceae, Cupress-aceae, and Taxodiaceae using exogenous applications of certain of the GAs would appear to be a promising technique for the forest geneticist and seed orchard manager, especially when used in conjunction with other promotive techniques *(e.g.* fertilization). The mechanism of exogenous GA action on the induction and/or promotive processes remains obscure, and additional, more basic research is necessary, not only to satisfy scientific curiosity, but for immediate practical reasons. For example, if a labor intensive technique using large amounts of an expensive hormone is to be economically practicable in seed orchard management, a thorough knowledge of the site of action, metabolism, ultimate fate, and most effective mode of application for the effector GA must be obtained. The possible role(s) of other plant hormones and/or nutritional factors should also be thoroughly explored relative to the efficacy of the GA effector. Fortunately, the recent chemical synthesis of radioactive GAs of high specific activity by Drs. RC. Durley and N. Murofushi at the University of Calgary will make this task much simpler. Even so, much research of both a basic and applied nature remains to be accomplished.

# LITERATURE CITED

Doorenbos, J. 1965. Juvenile and adult phases in woody plants. En-eye!. Plant Physio!. 15: 1222-1235.

Ebell, L. F. 1972a. Cone-induction response of Douglas-fir to form of nitrogen fertilizer and time of treatment. Can. I. For. Res. 2: 317-326.

Ebel, L. F. 1972b. Cone-production and stem growth response of Douglas-fir to rate and frequency of nitrogen fertilization. Can. I. For. Res. 2: 327-338.

Ebell, L. F. and E. E. McMullan. 1970. Nitrogenous substances associated with differential cone production responses of Douglas-fir to ammonium and nitrate fertilization. Can. J. Bot. 48: 2169-2177.

Eis, S., E. H. Garman, and L. F. Ebell. 1965. Relation between cone production and diameter increment of Douglas-fir *(Pseudotsuga menziessii* [Mirb.] Franco), grand fir *(Abies grandis* [Doug!.] Lindl.) and western white pine *(Pinus monticola* Doug!.). Can. J. Bot. 43: 1553-1559.

Evans, L. T. 1971. Flower induction and the florigen concept. Ann. Rev. Plant Physiol. 22: 365-394.

Fielding, I. M..1960. Branching and flowering characteristics of Monterey pine. Austr. For. Timber Bur. Bull. No. 37. 59 p.

Garner, W. W. and H. A. Allard. 1920. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. I. Agric. Res. 18: 553-606.

Giertych, M. 1967. Analogy of the difference between male and female strobilus in *Pinus* to the differences between long- and short-day plants. Can. J. Bot. 45: 1907-1910.

Giertych, M. 1972. Relation between nutrition, growth and flowering in coniferous trees. Second annual report. Inst. Dendr. and Kornik Arboretum, Polish Acad. Sci., Kornik, Nr, Poznan. 32 p.

Glenn,I. L. 1973. investigations concerning the physiology of gibberellin-induced growth and sexual differentiation phenomena in Arizona cypress *(Cupressus arizonica* Greene) seedlings. Ph.D. Thesis, Univ. Calgary, Calgary, Alta., Can.

Goo, M. 1968. Photoperiod and flowering of *Pinus densiflora* seedlings. Tokyo Univ. For. Misc. Inf. Ser. 17: 101-104.

Hashizume, H. 1959. The effect of gibberellin upon flower formation

in *Cryptomeria japonica.* J. Jap. For. Soc. 41: 375-381.

Hashizume, H. 1966. Chemical regulation of flower-bud formation and flower-sex differentiation in conifers. Chemical Regulation of Plants 1: 148-156.

Hashizume, H. 1967. Experimental induction of female flowers in young Japanese larch *(Larix leptolepis* Gordon). J. Jap. For. Soc. 49: 405-408.

Hashizume, H. 1969. Auxins and gibberellin-like substances existing in the shoots of conifers and their roles in flower bud formation and flower sex differentiation. Bull. Tottori Univ. For. 4: 1-46.

Hashizume, H. 1973. Studies on flower bud formation, flower sex differentiation and their control in conifers. [in Japanese, English abstract]. Bull. Tottori Univ. For. 7: 1-139.

Hillman, W. S. 1969. Photoperiodism and vernalization, p. 557- 601. *In* M. B. Wilkins (ed.), Physiology of plant growth and development. McGraw-Hill, London.

Jackson, D. l. and G. B. Sweet. 1972. Flower initiation in temperate woody plants. Hort. Abstr. 42: 9-24.

Kamienska, A., R. P. Pharis, R. L. Wample, C. C. Kuo, and R. C. Durley. 1973. Occurrence and metabolism of gibberellins in conifers. 8th Int. Conf. Plant Growth Substances. Tokyo, Jap. In press.

Kato, Y., N. Fukuharu, and R. Kobayashi. 1958. Stimulation of flower bud differentiation of conifers by gibberellin, p. 67-68. Abstr. 2nd Meeting Jap. Gibberellin Res. Assoc.

Kato, Y., N. Fukuhara, and R. Kobayashi. 1959. Stimulation of differentiation of flower bud in conifer by gibberellin (I) [in Japanese]. Nippon Ringakkai-Shi 41: 309-311.

Kroes, H. H. 1970. A study of phytochrome, its isolation, structure and photochemical transformations. Unilever Res. Lab., Duiven, Netherlands, 294th Comm. 112 p.

Kuo, C. C. 1973. Growth retardation and nutritional stress in relation to vegetative growth and reproductive differentiation of seedlings of *Cupressus arizonica* Greene. Ph.D. Thesis, Univ. Calgary, Calgary, Alta., Can.

Lang, A. 1965. Physiology of flower initiation, p. 1380-1536. *In* W. Ruhland (ed.), Handbuch der Pflanzenphysiologie, vol. 15/1. Springer-Verlag, Berlin-Heidelberg-New York.

Lang, A. 1970. Gibberellins: structure and metabolism. Ann. Rev. Plant Physiol. 21: 537-570.

Longman, K. A. 1960. Factors affecting flower initiation in certain conifers. Proc. Linn. Soc. Land., 172: 124-127.

Lyr, V. H. and G. Hoffmann. 1964. Uber den einfluss der mineral-salz-ernahring auf der fruhfruktifikation von *Cryptomeria japonica* (L.F.G.) Don. Flora 154: 189-208.

Owens, J. N. 1969. The relative importance of initiation and early development in cone production in Douglas-fir. Can. J. Bot. 47: 1039-1049.

Owens, J. N. and R. P. Pharis. 1967. Initiation and ontogeny of the microsporangiate cone in *Cupressus arizonica* in response to gibberellin. Am. J. Bot. 54: 1260-1272.

Owens, J. N. and R. P. Pharis. 1971. Initiation and development of western redcedar cones in response to gibberellin induction and natural conditions. Can. J. Bot. 49: 1165-1175.

Pharis, R. P. and C. C. Kuo. 1975. Physiology of gibberellins in conifers. Submitted to Can. J. For. Res.

Pharis, R. P., C. Kuo, and J. L. Glenn. 1972. Gibberellin, a primary determinant in the expression of apical dominance, apical control and geotropic movement of conifer shoots, p. 441-448. *In* D. J. Carr (ed.), Plant growth substances. Springer-Verlag, New York.

Pharis, R. P. and W. MorE. 1967. Experiments on the precocious flowering of western redcedar and four species of *Cupressus* with gibberellin $A_3$ and Am mixture. Can. J. Bot. 45: 1519-1524.

Pharis, R. P. and W. MorE. 1968. Physiology of gibberellin-induced flowering in conifers, p. 1341-1356. *In* F. Wightman and G. Setterfield (eds.), Biochemistry and physiology of plant growth substances. Runge Press, Ottawa.

Pharis, R. P. and W. MorE. 1969. Precocious flowering of coastal and giant redwood with gibberellins $A_4$, Am and $A_{13}$. Bioscience 19: 719-720.

Pharis, R. P. and W. MorE. 1970. Sexuality in conifers: effects of photoperiod and gibberellin concentration on the sex of gibberellin-induced strobili of western redcedar *(Thuja plicata* Donn.) Zesy. Nauk, Univ. Mikolaja Kopernika W. Toruniu 23: 85-89.

Pharis, R. P. and W. MorE. 1972. Short day and cold as causative factors in the anthesis-like development of strobili of western redcedar *(Thuja plicata)*. Can. J. Bot. 50: 2683-2685.

Pharis, R. P., W. Morf, and J. N. Owens. 1969. Development of the gibberellin-induced ovulate strobilus of western redcedar: quan-

titative requirement for long-day - short-day -long-day. Can. J. Bot. 47: 415-420.

Pharis, R. P. and J. N. Owens. 1966. Hormonal induction of flowering in conifers. Yale Sci. Mag. 41 (2): 10-19.

Pharis, R. P. and S. D. Ross. 1975. Promotion of flowering in the Pinaceae by gibberellins. II. Six-year-old Douglas-fir. Submitted to Physiol. Plant.

Pharis, R. P., M. D. E. Ruddat, J. L. Glenn, and W. Morf. 1970. A quantitative requirement for long day in the induction of staminate strobili by gibberellin in the conifer *Cupressus arizonica.* Can. J. Bot. 48: 653-658.

Pharis, R. P., M. D. E. Ruddat, C. C. Phillips, and E. Heftmann. 1965. Precocious flowering of Arizona cypress with gibberellin. Can. J. Bot. 43: 923-927.

Pharis, R. P., R. L. Wample, and A. Kamienska. 1974. Growth, development, and sexual differentiation in *Pinus,* with emphasis on the role of the plant hormone, gibberellin. *In* D. M. Baumgartner (ed.), Management of lodgepole pine ecosystems. Pullman, Washington. In press.

Puritch, G. S. 1972. Cone production in conifers. A review of the literature and evaluation of research needs (with an economic analysis by A. H. Vyse). Pac. For. Res. Centre, Can. For. Service, Victoria *B.C.* Information Rep. BC-X-65. 94 p.

Robinson, L. W. and P. F. Wareing. 1969. Experiments on the juvenile-adult phase change in some woody species. New Phytol. 68: 67-78.

Romberger, J. A. 1967. Flowering as a problem in developmental physiology, p. 2-14. *In* IUFRO-Kongress, vol. 14. Munchen, Germany.

Ross, S. D. and R. P. Pharis. 1973. Gibberellin-induced flowering of Douglas-fir grafts. Plant Physiol. 51 (Suppl.): 36.

Ross, S. D. and R. P. Pharis. 1975. Promotion of flowering in the Pinaceae by gibberellins. I. Sexually mature, non-flowering grafts of Douglas-fir. Submitted to Physiol. Plant.

Schmidtling, R. C. 1971. Cultivating and fertilizing stimulates precocious flowering in loblolly pines. Silvae Genet. 20: 220-221.

Smith, W. H. and R. N. Konar. 1969. Initiation of ovulate strobili in cotyledon-stage seedlings of *Pinus elliottii.* Can. J. Bot. 47: 624-626.

Sweet, G. B. and G. M. Will. 1965. Precocious male cone production associated with low nutrient status in clones of *Pinus radiata.* Nature 206: 739.

Vegis. A. 1964. Dormancy in higher plants. Ann. Rev. Plant Physiol. 15: 185-224.

Wample, R. L. and R. P. Pharis. 1975. Promotion of flowering in the Pinaceae by gibberellins. III. Seedlings of lodgepole *(Pinus contorta* Dougl.). Submitted to Physiol. Plant.

Wareing, P. F. 1959. Problems of juvenility and flowering in trees. J. Linnean Soc. Lond. 56: 282-289.

Wareing, P. F. and P. F. Saunders. 1971. Hormones and dormancy. Ann. Rev. Plant Physiol. 22: 261-288.

**End of Document**