14th Southern Forest Tree Improvement Conference

Sponsored by

THE SOUTHERN FOREST TREE IMPROVEMENT COMMITTEE

JUNE 14-16, 1977

GAINESVILLE, FL

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The Southern Forest Tree Improvement Committee is grateful to Mr. Merkel for contributing this motif symbolizing the purposes and aspirations of the Committee and its members.

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FORWORD

The Fourteenth Southern Forest Tree Improvement Conference was held in J. Wayne Reitz Union on the campus of the University of Florida in cooperation with:

The School of Forest Resources and Conservation
University of Florida

and

The Southeastern Forest Experiment Station
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SESSION I - TREE BREEDING

MODERATOR: ROBERT KELLISON

BREEDING BETTER URBAN TREES--
PROBLEMS, PRACTICES, AND POTENTIAL

Frank S. Santamour, Jr. 1/

Abstract.-- Importation of species or cultivars native to foreign countries has been, and will continue to be, an integral part of landscape-tree improvement in the United States. What is needed, in most exotic species, is a broader base of genetic variation from which superior selections and progenies can be developed. Seed source or provenance has been largely neglected in the past, but its importance is becoming recognized and appreciated. Still, the marketing practices of large commercial nurseries demand that only the most widely adaptable provenances will likely be recognized and propagated. Seed orchards are distinct possibilities for the continual production of superior trees in species where the need or desire for absolute uniformity is not great. The development of clones and cultivars based on single-tree selections will likely continue for the foreseeable future. Adequate testing of these cultivars will assure, that, in addition to the visual uniformity required in some landscape schemes, the cultivars will also be uniformly superior in survival traits. For cultivars propagated by budding or grafting, some attention must be paid to the provenance and adaptability characteristics of the rootstocks. Vegetative propagation is the norm in landscape-tree production and any improvement in rooting techniques or cell culture of difficult species could result in an abundance of new cultivars. Interspecific hybridization will continue to be important in developing new cultivars resistant to major disease and insect pests.

INTRODUCTION

Shortly after the research project on "Cytogenetics, Breeding, and Selection of Shade Trees" was established at the U.S. National Arboretum in 1967, I presented a comparative appraisal of the goals and procedures of tree improvement in horticulture and forestry to the Central States Forest Tree Improvement Conference (Santamour 1968). In subsequent papers and reports, I have attempted to amplify or clarify certain aspects of tree improvement for urban areas (Santamour 1969, 1971, 1972, 1976a). Today's presentation should be considered as merely another attempt to put urban tree improvement into an understandable perspective for scientists already trained in genetics and forestry.

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In that early paper, I wrote (somewhat hopefully) of an upsurge of interest in urban trees. The past decade has, indeed, seen an increased awareness of the plight of metropolitan flora and a modest increase in the number of scientists devoted to improving man's living and working environment through the production of superior woody plants.

The first International Symposium on the possibilities of genetic improvement of trees for metropolitan areas was held in 1975 (Santamour, Gerhold, and Little 1976). This Symposium was highlighted by the excellent spirit of cooperation among nurseryman, foresters, horticulturists, landscape architects, arborists, and representatives of associated scientific and practical disciplines. METRIA (the Metropolitan Tree Improvement Alliance) was both the parent and the child of the Symposium, and held its first conference in 1976.

There is no doubt that more and more persons are becoming interested in trees in urban areas. More often than not, positions of major responsibility are being filled by individuals with a forestry background. Therefore, in presenting this subject matter for the first time to the Southern Forest Tree Improvement Conference, I have followed the outline used by Dorman (1976) in chapter 20 of "The Genetics and Breeding of Southern Pines".

BUT FIRST, A FEW WORDS .....

As I talk with foresters around the country, I still meet some who are not totally aware of recent changes in the semantics and terminology of urban horticulture. There are many terms with various shades of meanings that could be discussed, but today I would like to focus on only a few.

The first of these is "ornamental". Does this word suggest useless, affluent, wasteful, superfluous, or other thesauric variations on the theme of non-utility? I think it does. It is a word that those of us engaged in the production, planting, and care of trees in urban environments cannot countenance. It is a word that is out of place in these times of ecological and environmental awareness. It is a word that should never have been used to describe so intricate an object as a living, green plant. So, let us erase "ornamental" from our lexicon, and concentrate on the ecological imperatives of plants for people.

What can we substitute for "ornamental"? The British have been doing quite well with "amenity" for a long period of time. The more ecologically aware have suggested "environmental". My own favorite, at least with regard to woody plants destined for outdoor use in city and suburb is "landscape". A landscape tree, a landscape shrub, a landscape planting: all phrases at least alluding to the interrelationship of plants and man. There may be other, and better substitutes for "ornamental". Whatever they are, let's use them.

The second term is "cultivar". Even though the International Code of Nomenclature for Cultivated Plants was published in 1969 (Gilmour and others 1969), it has not made much of an impact on forestry or foresters. However, as foresters move out of the woods and into the streets, they would do well to become aware of the Code and its ramifications. Basically, "cultivar" was coined from "cultivated variety" and was intended to replace the improper use of "variety" in designating vegetatively propagated plants selected from or intended for cultivation and which had no counterpart in natural plant communities. Those "fancy" names you see in nursery catalogs are all cultivar names, used to distinguish a particular selection from all other selections within a species or genus and especially from run-of-the-seedlot seedlings.

A "clone" may be a cultivar, but not necessarily. A cultivar need not be a clone. For a more provocative discussion of this latter point, see Santamour (1976c). But do get hold of a copy of the Code and see how it pertains to forestry and forest-tree improvement and read Dudley's (1976) paper in the aforementioned Symposium.

I could also speak of "arboriculture", which is the art and science of cultivating trees for the improvement of man's environment. The International Shade Tree Conference, the world's largest organization of arboriculturists, changed their name to the International Society of Arboriculture in 1975, in recognition of the provenciality of "shade tree" as compared to the more international "arboriculture".

What, then, is an "urban tree"; the subject of my talk today. Perhaps it would have been better to use "landscape tree" in the title, but with the current vogue of "urban forestry", the "urban tree" should have some status. I must confess that up until now I have not been pressed for a definition -- and it might be prudent to duck the question. An "interim" definition might state that an urban tree is one presently growing in, or destined for, environments characterized by a lack of natural tree vegetation and an abundance of people and people's things.

METHODS OF URBAN TREE BREEDING

IMPORTATION

Importation of species from foreign countries has been an important aspect of the nursery and landscape scene since the late 1700's. The European horsechestnut (Aesculus hippocastanum L.) was imported as seed in 1741, and it became the "spreading chestnut tree" under which Longfellow's "village smithy" stood. The Lombardy poplar (Populus nigra L. 'Italica' and Ginkgo biloba L. were introduced into this country in 1784, and the weeping willow (Salix babylonica L.) also came in the latter part of that century (Li 1963). Norway maple (Acer platanoides L.) made its first appearance in 1870 and the "London" plane (Platanus x acerfolia (Ait.) Willd.) in about 1900. More recent introductions include the Oriental cherries, Siberian elm (Ulmus pumila L.), Callery pear (Pyrus calleryana Dcne., and the Dawn redwood (Metasequoia glyptostroboides Hu & Cheng).

How many species native to foreign lands now are growing in the United States would be difficult to estimate. Indeed, one is tempted to think that practically all foreign tree or shrub species capable of survival in the more temperate zones have already been tried at least once. The "once" in the last sentence is a critical point.

The fact is, that even with the thousands of exotic species presently growing in this country, there may be little genetic diversity within species available for landscape-tree improvement research. Early, and sometimes the only, introductions of certain species were made from plants already under cultivation in their native lands. Furthermore, in some genera like Magnolia, it is possible that the wild progenitors of some cultivated species no longer exist in the native state.

We might already have the hardiest, most adaptable, most pest-resistant, and most floriferous specimens of a given species available to us. Or we might have only the "culls". Few studies have been made to determine the extent of available genetic diversity in non-forest trees of foreign origin. Feret and Bryant (1974) found that American sources of Ailanthus altissima (Mill.) Swingle (Chinese tree-of-heaven) possessed a considerable amount of variability as compared to native Chinese sources. Similar studies are desperately needed in more important exotic species.

Past plant exploration for hardy exotic trees was conducted largely along opportunistic rather than systematic lines, and with few exceptions, this method has persisted to the present day. It is likely that no single organization has the land, labor, money, interest, or tenacity to adequately develop a broad genetic base for the exotic species of even a single large genus, like the maples. The enormity of the task of assembling germplasm collections for the thousands of horticulturally important exotic species is sufficient to dissuade most arboreta, experiment stations, or nurseries from even making a start.

At the U.S. National Arboretum we have only recently begun efforts to develop domestic seed sources for some of the important Asiatic birch (Betula) species. Our interest in this project was prompted by the discovery that many Asiatic birches in the collections of American arboreta were not even true to species. We have made a good beginning with Japanese white birch (B. platyphylla Suk. var. japonica (Miq.) Hara and are also attempting a collection of monarch birch (B. maximowicziana Reg.) sources. Other exotic white-barked birches deserving of attention are B. costata Trautv. and B. jacquemontiana Spach. In addition, we are working to preserve the entire gene pool of the recently rediscovered native American B. uber Ashe (Ogle and Mazzeo 1976).

There is no doubt, however, that past introductions of non-native trees have served the nursery industry and the American public well over the last few decades. By prudent selection among well-adapted trees in street and landscape plantings or nursery plots, American nurseryman have produced vegetatively propagated cultivars of great utility and esthetic qualities, notably in Norway maple and flowering crabapples. Direct importation of cultivars developed by European and Asiatic nurserymen or arboreta has brought some fine plants to our shores and the products of

foreign research projects, like the disease-resistant elms from the Netherlands, may well play a major role in the future.

We should continue to exploit the variation encountered within existing American populations of exotic trees and strive to make further introductions of important foreign species more meaningful for future tree-improvement research. The key to the successful utilization of these foreign introductions, whether from 1777 or 1977, is, of course, testing.

RACIAL AND STAND SELECTION

Many nurserymen have, over the years, relied on certain trees or stands as parents for the production of seed-propagated species. Often the choice has been dictated merely by convenience, but the economics of production practices would argue that some selection for uniformity of seed germination, growth characteristics, and adaptability to nursery culture must have taken place. The selected trees or stands may or may not have been native to the region where they were growing but the fact that they were selected would indicate that they were at least well-adapted to that particular area. This type of seed source selection and use demonstrates, with few exceptions, the limited attention most nurseries have paid to geographic seed origin.

When the "selling range" of the nursery was restricted to a local area or within a given climatic zone, the seed-propagated progenies could be expected to perform reasonably well. When these progenies were sold and planted outside their zone of origin, the results were erratic and often disastrous. Still, there was a certain measure of protection against total failures by the built-in genetic variability of the seedlings.

As clonal and cultivar selections became more widely used in the nursery trade, the genetic variation was reduced, and thus the geographic origin was even more important. For many of the more widely used cultivars, the original seed source is unknown or relatively imprecise. One of the most popular red maple (Acer rubrum L.) cultivars was selected in Oregon from trees grown from seed collected "in Pennsylvania". Some cultivars of sweetgum (Liquidambar styraciflua L.), now being sold throughout the country for their attributes of fall color, were selected for this esthetic trait in California, after being grown there from seed of largely unknown origin.

The fact is that we know very little about the geographic origin of most of our cultivars of native trees and, perhaps, even less about the exact origin of exotic species. This lack of knowledge concerning origin has not deterred the widespread sale and use of many cultivars and few catastrophes have been reported that could be traced to improper provenance. Perhaps, if more exact information were available concerning the geographic origin of certain cultivars, the full impact of poor provenance choice could be determined.

What are nurserymen doing about provenance selection? What are the arboreta and agricultural experiment stations doing about provenance selection? What are the handful of landscape-tree geneticists doing about provenance? The answer is "very little".

Forest geneticists in forestry-oriented institutions are virtually the only scientists actively exploring the genetic variation in broad-leaved deciduous trees, and this testing is largely with native American species. Many of our important oak and poplar species are being studied, as well as sweetgum and tuliptree, white and green ash, American sycamore, sugar and red maples, yellow and paper birches, etc. These tests may vary in their breadth and purpose, but most of them are designed for limited utility and the test areas cover only a small portion of the "selling range" of our major nurseries -- the 48 contiguous States.

What kind of impact will the results of such provenance studies have on the commercial nursery trade? The more progressive nurserymen will be interested, of course, since they have a personal commitment to grow the best plants possible. But can they afford to custom-grow plants for the diverse geographic or climatic zones of adaptation? I doubt it. If one or a few provenances can be singled out as having a rather broad range of adaptability, these would be the sources of greatest interest to nurserymen. Then, provenance research in "forest" species will have a positive influence on the landscape-tree business.

It should be mentioned here that for budded or grafted cultivars, the provenance of the rootstock may be as important (or more so) as that of the scion. Flemer (1976) has pointed out the necessity of cold-hardy rootstocks for trees destined for growing in containers in northern areas. The use of rootstocks of more northern origins may well extend the usable range of some trees that have a restricted geographic use on their own roots or on roots of seedlings from more southerly origins. The importance of rootstock provenance and the rootstock influence on the adaptability and performance of the scion cultivar are neglected areas of research.

SINGLE-TREE SELECTION

Single-tree selection is the very backbone of landscape-tree development. Nurserymen have, since the beginning, selected and propagated those trees that differed significantly from the "average" tree of a particular species. Unfortunately, much of this selection was for only visual and visible traits. Thus we have an abundance of dwarf, prostrate, weeping, creeping, and crawling cultivars with red, yellow, and variegated leaves gracing our nurseries, arboreta, cemeteries, streets, parks and houselots. The most bizarre cultivars have been selected among coniferous species, In most broadleaved deciduous species, the selection criteria of growth habit, growth rate, leaf texture, flower color, etc. have resulted in more "normal" trees. Some of these cultivars may be fine plants, with a wide range of adaptability and resistant or tolerant on the major biotic and abiotic stresses of urban areas. If there are such cultivars, we are indeed fortunate.

Is clonal or cultivar uniformity more dangerous than species uniformity? We tend to think so. Of course, the American chestnut (Castanea dentata Borkh.) had all of its genetic diversity available to withstand the charge of the chestnut blight. It lost!

Single-tree selection and the vegetative propagation of selected individuals will continue in landscape-tree research and development. It just makes good sense. The key to the success of this method in the future is testing: for climatic adaptability, for tolerance to air pollution and salty or compacted soil, for resistance to major insect and disease pests, and for the ability to recover from injury. We hope that, with the rather recent entry of a few scientists into the field of landscape-tree improvement, that nurserymen will be made more aware than ever of the need for thorough and rigorous testing. And we hope that we, as scientists, can practice what we preach.

SEED ORCHARDS

The concept of the "seed orchard" is, I think, relatively new to horticulture. Going back to my comments on seed source, it is likely that some nurserymen, when asked, might consider their parent tree or stand as a "seed orchard". They may be right--partially--but again the key word is testing: The testing (and selection) that turns a bunch of trees into a bona fide seed orchard.

The products of a seed orchard are superior trees. In current forestry practice there is, as yet, no uniformity in the designation of these superior seedlings. The superior seed or seedlings may be certified or the source described in some "longhand" fashion.

In landscape-tree horticulture, we have the advantage, I think, of being able to use the "shorthand" of a "fancy" cultivar name to refer the superior trees produced by the seed orchard method. Remember that the Code does not restrict the designation of cultivar to vegetatively propagated plants.

At the moment, there are very few seedling cultivars of woody plants destined for landscape or non-forest use. One of the more famous, deserved so, is the 'Chinkota' Siberian elm developed in South Dakota for planting in windbreaks and shelterbelts in the Plains States (Collins 1955). This cultivar is the result of intensive selection for cold hardiness and climatic adaptability and has been produced under controlled conditions for over 20 years.

The use of cultivar names to designate the products of seed orchards is not, and should not be, restricted to non-forest trees. Forest trees are cultivated plants and, as such, come under the broad aspects of the Code. It is time that forest-tree geneticists recognize their responsibility to the Code and adapt its tenets-- or at least devise a similar system whereby seed and seedlings from seed orchards can be named, numbered, and recognized.

At the present time, commercial nurserymen grow trees from seed for two reasons: (1) it may be cheaper, or (2) the species cannot be easily propagated by vegetative means. Whether it is less expensive to grow seedlings as compared to vegetatively propagated selections is a matter for some argument, but it is noteworthy that most new, marginal, or fly-by-night nurseries offer only seedlings.

Not all landscape-tree species are suited to production in seed orchards -- at least not with our present state of knowledge. The inheritance of resistance to disease, such as Dutch elm disease or sycamore anthracnose, is seldom so dominant that the seedlings could be "guaranteed" resistant. The current vogue of using only male plants of dioecious species (e.g. Ginkgo biloba, Fraxinus americana L.) also argues against the seed orchard concept. There are also variables such as flower color, tree form, and leaf coloration that might not be as uniform in a seedling cultivar as in a grafted or rooted cultivar.

However, when we learn more about the genetics of our landscape trees and also learn that we can live (perhaps better) without the high degree of uniformity that we have come to expect from vegetatively propagated cultivars, we will see a dramatic increase in the use of seed orchards as an improvement method for woody plants in horticulture. Seed orchards will not replace single-tree selection and propagation, but should provide an important alternative for many landscape species.

INTERSPECIFIC HYBRIDIZATION

Many of our most important present-day cultivars (e.g. "London" plane, crabapples) are interspecific hybrids that occurred in nature or accidentally, under cultivation. To some persons, genetics is hybridization; and to a large segment of the public, the word "hybrid" denotes some superior status.

At the National Arboretum, we have been very active in hybridization research because (1) it is a rapid means of producing diversity in an often inadequate gene pool, (2) it gives meaningful clues to species and generic relationships, and (3) it is frequently the only means of transferring certain desirable characteristics (such as disease resistance) to a widely used and adaptable species.

Forest-tree improvement in the United States has tended to rely, rightly, on well-adapted native species. Exotic species, whether as replacements for native species or as parents in hybrid combinations with natives have not proved to be very important. This should not be surprising, since the wide range of genetic diversity, (quite readily available) allows for critical selection procedures to solve most problems.

Perhaps it was the unavailability of intraspecific genetic diversity that has led most horticultural geneticists down the path of interspecific hybridization. If so, that is one very valid reason. Another reason might be that the rare "super" gene combinations developed by hybridization could be put to immediate use through vegetative propagation. Whatever the reason, I would predict that interspecific hybridization will continue to play an important role in landscape-tree improvement and also, as forestry becomes more intensive, that hybrids may solve some of the problems that will be encountered in such mass cultures.

It is interesting to note that, in the Netherlands, the new hybrid cultivars -- the only ones to show adequate resistance to the "aggressive" strains of the Dutch elm disease fungus -- have a "touch" of Himalayan elm (Ulmus wallichiana Planch.) in their background (Heybroek 1976). If this research project had relied exclusively on the European elm complex, which had produced four very fine cultivars, the appearance of the "agressive" strain might have not only wiped out the elms but also the research.

VEGETATIVE PROPAGATION

Vegetative propagation has been the cornerstone of plant improvement in horticulture for hundreds of years, and yet we still do not have all the answers. If, at this meeting, a paper were presented which outlined a "foolproof" technique for rooting stem cuttings of oaks, I am sure that, within 5 years there would be 50 new oak cultivars on the market. Would such a development be good or bad? Overall, I think it would be good. There are many apparently superior oak genotypes presently on our city streets and we cannot even test them, let alone mass-produce them, without an efficient means of vegetative propagation. The glut of new cultivars in any genus with landscape potential has always caused problems, but time and adequate evaluation should separate the "winners" from the "also rans".

It is perhaps true, however, that there is no species of woody plant that is impossible to root from cuttings. Not too long ago, the idea of establishing forests of clonally propagated pines and spruces seemed out of the question. The technology to produce such forests is now at hand.

We still need better rooting or cell-propagation techniques for difficult species. We need a greater understanding of the causes of graft incompatability. We need to know how and how much the rootstock influences the behavior of selected scion cultivars. The problems are solvable, if enough research effort is expended.

POLYPLOIDY AND MUTATION BREEDING

The use of colchicine to induce polyploidy and ionizing radiation and other agents to produce mutations has found little utility in forest-tree improvement. The same may be said of landscape-tree improvement. While such studies are of special experimental interest, the production of superior trees by these techniques may be difficult, and rather unlikely, in many genera.

However, in this context I would like to mention a rather effective new approach to woody plant improvement presently being explored at the National Arboretum. As mentioned previously (Santamour 1976b), the lack of fruit production can be a desirable attribute for many landscape trees and shrubs. Dr. Donald R. Egolf, at the Arboretum, has pioneered the use of colchicine-induced tetraploids in crossing to normal diploids to produce sterile (fruitless) triploid plants. The individual flowers of "created" triploids in Hibiscus syriacus L. remain on the plant longer and the entire flowering period is lengthened. The lack of seed eliminates the potential problems that may occur when unwanted seedlings germinate in cultivated soil near the plants. It is also likely that the lack of fruit and seed production actually enhances the vigor and adaptability of the plant, although no studies have been made. Only one Hibiscus cultivar developed by this technique has been released so far (Egolf 1970), many other triploid selections of Hibiscus and Lagerstroemia are under evaluation.

Sterility and lack of fruit production are, of course, not the same thing. Parthenocarpy in such self-incompatible genera as Liquidambar and Liriodendron might eliminate any supposed advantages of triploidy in eliminating fruit production. Still, there are a number of genera like Albizia, Catalpa, Paulownia, and Sophora in which we have attempted the first step (induction of polyploidy by colchicine treatment) to test this technique.

It is also possible that triploids created by interspecific crosses in genera having polyploid series (Carya, Fraxinus, Tilia) may be fruitless.

LITERATURE CITED

Collins, Paul E. 1955. Chinkota elm. S. Dak. Farm and Home Res. 7(1): 14-16, 27.

Dorman, Keith W. 1976. The genetics and breeding of southern pines. USDA Forest Serv. Agric. Hndbk. No. 471, 407 p.

Dudley, Theodore R. 1976. How to name, register, and patent selected cultivars of amenity plants. In Better trees for metropolitan landscapes, USDA Forest Serv. Gen. Tech. Rpt. NE-22, 235-243.

Egolf, Donald R. 1970. Hibiscus syriacus 'Diana', a new cultivar. Baileya 17(2): 79-82.

Feret, Peter P. and Bryant, Richard L. 1974. Genetic differences between American and Chinese Ailanthus seedlings. Silvae. Genet. 23: 144-148.

Flemer, William, III. 1976. Container trees for use in landscaping. In Better trees for metropolitan landscapes, USDA Forest Serv. Gen. Tech. Rpt. NE-22, p. 185-193.

Gilmour, J.S.L. and others. 1969. International code of nomenclature of cultivated plants. Regnum Vegetabile 64, 32 p.

Heybroek, H.M. 1976. Three new clones of elm. Nederl. Bosb. Tijds. 48(5): 117-123 (In Dutch).

Li, Hui-Lin. 1963. The origin and cultivation of shade and ornamental trees. Univ. Penna. Press, 282 p.

Ogle, Douglas W. and Mazzeo, Peter M. 1976. Betula uber, the Virginia round-leaf birch, rediscovered in southwest Virginia. Castanea 41: 248-256.

Santamour, Frank S., Jr. 1968. The forest and the street: A comparison of tree improvement in forestry and horticulture. Central States Forest Tree Impr. Conf. Proc. 6: 1-5.

Santamour, Frank S., Jr. 1969. Breeding trees for tolerance to stress factors of the urban environment. Second World Consultation on Forest Tree Breeding, Document FO-FTB-69-6/4, 7 p.

Santamour, Frank S., Jr. 1971. Shade-tree improvement research: Goals and limitations. Northeast. Forest Tree Impr. Conf. Proc. 17: 60-64 (1969).

Santamour, Frank S., Jr. 1972. Developing shade trees for our urban areas. In Landscape for Living, USDA Yrbk. p. 16-22.

Santamour, Frank S., Jr. 1976a. Breeding and selecting better trees for metropolitan landscapes. In Better trees for metropolitan landscapes, USDA Forest Serv. Gen. Tech. Rpt. NE-22, p. 1-8.

Santamour, Frank S., Jr. 1976b. Perspectives on improvement strategies for metropolitan trees. In Better trees for metropolitan landscapes USDA Forest Serv. Gen. Tech. Rpt. NE-22, p. 215-220.

Santamour, Frank S., Jr. 1976c. Clone vs. cultivar: The root of the problem. Amer. Nurseryman 144(4): 20,36.

Santamour, Frank S., Jr., Gerhold, Henry D., and Little, Silas (Eds.) 1976. Better trees for metropolitan landscapes, USDA Forest Serv. Gen. Tech. Rpt. NE-22, 256 p.

ROOTED CUTTINGS IN PRODUCTION FORESTS

W.J. Libby[1/]

Abstract.--This paper presents a case for using rooted cuttings in forestry. It briefly discusses their advantages and disadvantages with respect to genetics and breeding, forest ecology, and management. Hedging and other techniques are discussed as ways to control the maturation state of the clones. A major effort will be needed to adapt nursery technology to rooted cuttings.

Additional keywords: Competition, clonal selection, diversity, hedging, juvenility, specific combining ability.

INTRODUCTION

In order to take the first parts of this paper seriously, you must (at least provisionally) accept two assumptions: (1) Rooted cuttings survive and grow as well as or better than seedlings. (2) Rooted cuttings can be produced and planted at costs similar to those for seedlings. These assumptions will be discussed near the end of this paper.

GENETIC ADVANTAGES OF ROOTED CUTTINGS

There are impressive genetic advantages associated with the use of rooted cuttings. The first usually thought of concerns non-additive genetic variability, or specific combining ability. For many characteristics in many species, much of the genetic variation appears to be additive. This is fortunate, as most of our present breeding schemes and production seed-orchards are based on the general combining ability associated with additive genetic variation. But in some cases, a significant component of the genetic variation is non-additive. While some schemes have been proposed to utilize this kind of variation (such as 2-clone orchards), few are now operational. Clonal testing of rooted cuttings will allow us to identify those occasional individual genotypes that are outstanding due to a particular non-additive combination of genes. This outstanding performance will not be consistently repeated by either the sibs of such outstanding trees (the pure lines necessary for such sib consistency are not yet generally available in forestry) or by their offspring. This outstanding performance will be consistently repeated by rooted cuttings of these specific outstanding genotypes.

Inbreeding depression is another manifestation of non-additive genetic variation. It affects the offspring when relatives mate or are mated. Reductions in growth and vigor have been reported for most of the forest-tree species that have been subjected to controlled inbreeding. Inbreeding depression may be common among seedlings collected from those wild stands with a population structure that makes pollination by relatives likely. It will

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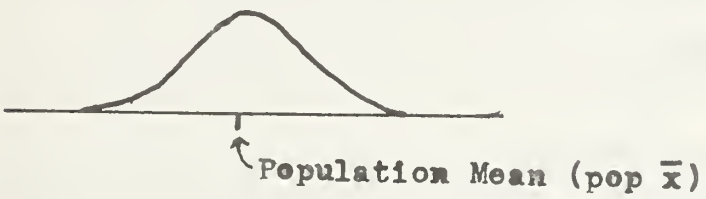
also occur in seed-orchards that incorporate relatives. It is particularly likely in seed-orchards where more than one sib of each selected family is used to produce seed. If the normal reforestation propagule is a rooted cutting, those genotypes (seedlings or clones) exhibiting inbreeding depression can be excluded, and the production plantations can thus be free of inbreeding depression.

There is an important advantage to rooted cuttings associated with additive genetic variation, or general combining ability. Pick your favorite characteristic or index, and please consider the following:

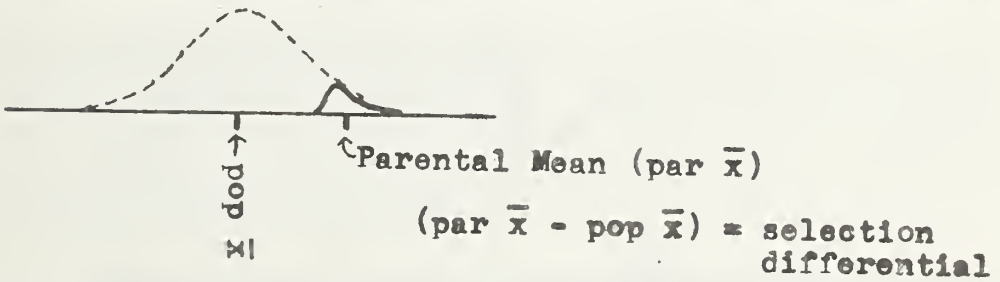
Phenotypic variability of the original seedling-origin population may be distributed something like:

A.



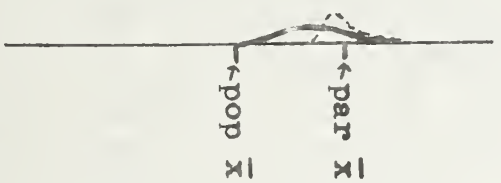
From this population, we select the following phenotypes as parents:

B.



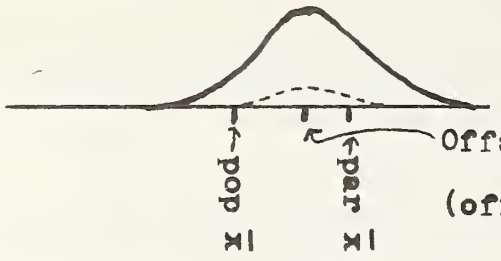
The phenotypes of these selected parents are partly the result of favorable enviroments and favorable non-additive genetic combinations. The "additive-genetic" genotypes of the selected parents may be distributed something like:

C.



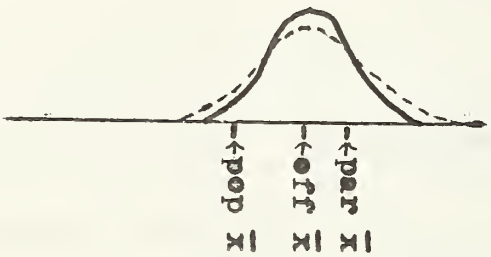
The phenotypes of their offspring will be distributed something like:

D.



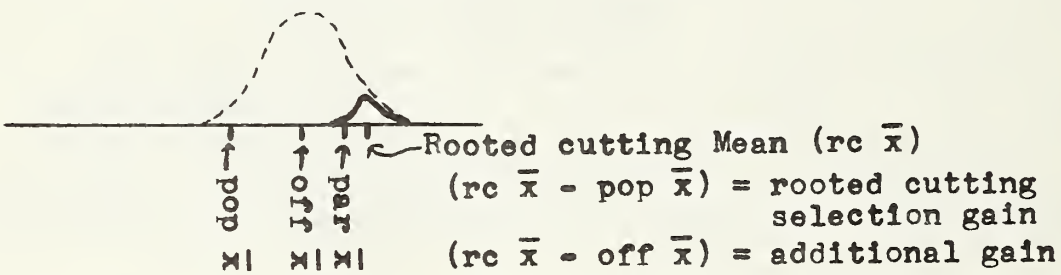
Because of sexual recombination of the parental genes, the offspring genotypes will exceed the range of the parental genotypes, and, with environmental contributions to variation averaged out, will be distributed something like:

E.



If we use rooted cuttings of only the best of these offspring, depending on how effective our clonal selection is, the genotypes of the selected clones will be distributed something like:

F.



This substantial additional gain is possible because half or more of the additive genetic variance occurs within families, and a tree-improvement program relying on sexually produced seedlings can only take advantage of the average performance of its selected families. By applying clonal selection and using rooted cuttings of the very best individuals in selected families, we can take advantage of the remaining half (or more) of the genetic variation thus available to us. This may require some cost in time for the additional selection. However, it can and should be done incrementally, by first eliminating those clones which are deformed or are runts, or otherwise disqualify themselves, and then periodically eliminating other clones that develop characteristics that don't measure up. Thus, within-family clonal selection can begin in the very first year that pedigreed seedlings and cloning techniques are available, and it should continue to refine the donor population as information on the clones accumulates through rotation age and beyond. Even in the first year, the average performance of the rooted cuttings should equal or exceed that of seedlings from a seed-

orchard (Figure D, above), and as the number of clones under test and in use is selectively reduced from (say) 10,000 to 500, the additional gain of the rooted cuttings will approach that shown in Figure F.

In some species, many of the very best-growing trees produce few seeds and/or little pollen in seed-orchards. On the other hand, the offspring of the unusually prolific pollen- and seed-producers in the seed-orchards could repeat this sexual performance in production forests, thereby reducing average stand growth. The principle in both cases is that photosynthate devoted to sex is usually at the expense of wood production, and there is some evidence that sexiness in heritable in trees. With rooted cuttings, those genotypes that devote little energy to sex can be encouraged to produce modest numbers of offspring in at least an occasional favorable year, and some of these can be added to the set of cutting donors and put under test. Thus, while the genetic contribution of genotypes that concentrate on making wood (not love) may be small or even absent in some years in a sexually-reproducing seed-orchard, they can contribute their appropriate share of rooted cuttings every year.

Gene conservation is a genetic consideration of some importance. Clonally maintaining an appropriate sample of unselected populations may be an effective way to conserve a safe supply of future variability, without contamination by pollen from our increasingly-domesticated production forests.

GENETIC DISADVANTAGES OF ROOTED CUTTINGS

We have found that clones age, and that later cuttings are different from earlier cuttings of the same clone in such properties as growth-vigor and form. Knowing this, it has been considered futile to try to identify good clones if the clones continue to mature and thus change their properties during the test period.

In a breeding program, some selection differential is sacrificed in applying clonal selection (where each genotype is replicated over the test environments), compared to selection in a system using strictly seedlings (where each genotype appears only once). We need more theoretical work and more practical experience to help us assess the various trade-offs (such as selection accuracy, and assessment of genotype-environment interaction) against this loss of selection differential.

ECOLOGICAL ADVANTAGES OF ROOTED CUTTINGS

I now think that the most attractive feature of using rooted cuttings is the possibility of maintaining high genetic diversity within production forests. This results from two kinds of control available with clonal propagation. The first is pedigree control on each clone, so that one can avoid having related clones in the same production plantation. The second is that each clone is a separate biotype. Thus, although an insect or pathogen may be closely adapted to one clone in a plantation, there will be a genetic gap between clones. By contrast, seedlings from a seed-orchard are a continuum of biotypes, with a greater possibility of incremental adaptation of the insect or disease across the entire population being used in plantations. Furthermore, since it appears economically feasible to genetically improve more species with rooted cuttings than with seedlings (see

below), improved mixed-species plantings are also more feasible using rooted cuttings.

Along this same line, it is possible to manage rooted-cutting reforestation so that it is unlikely that any two plantations will have exactly the same set of clones. As an example, the project computer might have information on the known performance of (say) 2,000 good clones appropriate to a particular region. A forester would request planting stock for a plantation, and provide information on such things as latitude, longitude, elevation, soil, aspect, pest history, etc. The computer would print out the identity of the 50 best clones for such a site. These would then be planted in mixture. A request for another plantation site in that region would produce another list of 50 clones, 15 of which might be the same as for the first site, but 35 different. By contrast, if the same regional seed-orchard were serving both sites, each plantation would receive seedlings that were very similar samples of the same general seed-orchard production.

As we learn a great deal about these clones, it may be possible to prescribe not only favorable planting mixtures, but favorable planting sequences. In particular, clones making complementary (rather than competing) demands on the site would be planted next to each other.

ECOLOGICAL DISADVANTAGES OF ROOTED CUTTINGS

There is a danger of selecting too few clones per plantation, the extreme being a monoclonal planting. Then if some event occurs, such as a cold snap or a disease, an unacceptable proportion of the plantation may be damaged or lost. Note that this is not an intrinsic disadvantage of rooted cuttings, but rather, is a potential for management error.

MANAGEMENT ADVANTAGES OF ROOTED CUTTINGS

There are many problems associated with seed-orchards that can be avoided by using rooted cuttings. These include graft incompatibilities, pollen contamination, cone and seed pests, and a short cone-collection season. Economies of scale can be achieved by growing cutting-donors serving many different kinds of sites together. This should not be done with seed-orchards if there is a possibility of cross-pollination between trees from the different kinds of sites. Similarly, cutting-donors may be grown on land surrounded by forests of the same species, but pollen contamination can force the location of seed-orchards to areas far removed from other management activities.

With clones, the single-family pedigree is the unit of selection, and each cutting-donor is essentially independent of all others. Because of the flexibility this provides, relatively minor species and unusual sites can be served by a modest number of selected, known, and appropriate clones. By contrast, a special seed-orchard is unlikely to be economically justified for each specialized relatively minor demand.

Finally, known clones will have more predictable performance than will seedlings, each of which is brand new on the face of Earth. Aggressive management should be able to use this greater predicability in many ways.

MANAGEMENT DISADVANTAGES OF ROOTED CUTTINGS

Producing a healthy, plantable rooted cutting is not the same as producing a seedling. It will be necessary to develop many new nursery techniques, and this will take both time and money.

THE COMPARABILITY ASSUMPTION

In most species, cuttings taken from late-adolescent or mature trees do not grow with the same form and vigor as do seedlings. While some aspects of form are better in such rooted cuttings, the cuttings are almost always distinctly inferior to seedlings in both early survival and volume growth. However, cuttings taken from young seedlings are very similar to seedlings in survival, development, and growth. In other words, the growth and development of a rooted cutting is very much dependent on its maturation state.

The trick, then, is to keep some members of each clone in a juvenile stage of maturation, not only until other members of the clone can be evaluated, but for a much longer time so that identified superior clones can be extensively employed. There now appear to be several ways to accomplish this trick. My current favorite is called "hedging", which means that a seedling or rooted cutting is repeatedly clipped back, thus forming it into a hedge. Cuttings taken from such repeatedly hedged donors are juvenile, even though the chronological age of the hedge is well beyond a juvenile age. We have done this for several years with Monterey pine (Pinus radiata), and are now engaged in parallel studies with about a dozen species. Several other research centers are also engaged in such studies with many other species. The early results are encouraging, but for most species we won't know how long clones can be held in a juvenile state until we've actually done the experiments for those periods of time.

Another method being used to arrest maturation is serial propagation--i.e., cuttings are taken from recently-rooted cuttings, which were taken from recently-rooted cuttings, etc. And, several research centers are investigating ways of reversing maturation, so that tissue from an outstanding mature tree can be returned to a juvenile state, and a juvenile clonal line of that genotype can thus be established. However, there are as yet no reliable techniques for doing this.

It is possible that we may not want completely comparable maturation states of cuttings and seedlings. For instance, early-adolescent cuttings of radiata pine have attractive advantages with respect to resistance to two important diseases, and have better stem-form than seedlings (or more juvenile cuttings), with little or no difference in relative growth rates. Thus, the preferred propagules for radiata pine reforestation may be rooted cuttings at maturation state of about 4-8 years, rather than either seedlings or juvenile cuttings. It appears that we may be able to use hedging to set and hold any maturation level we desire. Indeed, we have held over 200 clones of radiata pine at about a 4-year-old maturation state for 7 years, from 1965 to 1971, by hedging at a height of about one meter.

THE PRODUCTION ASSUMPTION

With exceptions in a few species, a plantable rooted cutting is more expensive than a plantable seedling. For some species, this may always be true. For others, it may just require creating a new technology of rooted-cutting nursery practice. When rooted-cutting research and experience is similar to that for seedlings, so may be its relative costs. If the advantages of rooted cuttings over seedlings are sufficient, absolute equality of production costs need not be achieved in order to decide to use cuttings. For example, the additional cost of vegetatively-propagated fruit trees (compared to seedlings) is easily justified. In the tree-fruit industry, seedlings are essentially limited to use in breeding programs, with selected new genotypes clonally propagated to production plantings.

A PREDICTION

Within the next 10-15 years, in a substantial number of forest-tree species now planted exclusively as seedlings, the majority of production plantations will routinely include rooted cuttings of selected clones in their planting stock.

INFORMATION ON RECENT WORK

Much recent thinking and information on rooted cuttings of forest trees is available in the proceedings of 2 symposia: the 1973 meeting on vegetative propagation of forest trees; and the 1975 meetings on juvenility in woody plants (both cited below).

LITERATURE

1974. Special Issue on Vegetative Propagation. New Zealand Jour. of Forestry Science 4(2):119-458.

1976. Symposium on Juvenility in Woody Perennials. Acta Horticulturae 56 (May):1-317.

EXTRACTION AND STORAGE OF LOBLOLLY PINE (*Pinus taeda*) POLLEN

Jerry R. Sprague and Vernon W. Johnson[1/]

Abstract.--Five years of investigations at North Carolina State University on extraction and storage of loblolly pine pollen have revealed the following: (1) the most critical factor in pollen storage is keeping the catkins and pollen dry during the extraction process; (2) pollen dried to a moisture content of 8 to 10 percent will store satisfactorily for two to three years with most of the methods investigated; (3) the vacuum method of pollen storage is effective for up to three years and yields higher and more consistent viability than do the conventional methods tested. Justification of this expensive method is still in question at this time.

Additional keywords: Moisture content, vacuum storage, germinability, catkins, clonal variation.

Due to the fact that it takes one to two weeks to collect and extract southern pine pollen, the use of pollen for control-pollinations during the same year of collection is very difficult. Therefore, pollen storage for use of pollen in subsequent years has been employed by tree breeders. To insure the highest viable pollen for control-pollinations, proper extraction and storage procedures must be determined. In the past the North Carolina State University-Industry Cooperative Tree Improvement Program has advised its members to extract pollen and dry it over a desiccant (usually LiCl) under refrigerated conditions at approximately 35°F (2°C). However, success with this method seemed rather sporadic. At the same time, the Cooperative decided to establish a breeding pollen bank at North Carolina State to facilitate the control crossing in the advanced-generation breeding. Therefore, it was decided to initiate investigations to determine the best methods of pollen extraction and storage for insuring the most viable pollen. Investigation began in the spring of 1973. This paper reports observations and results from investigations in four areas: (1) pollen extraction; (2) pollen storage; (3) effectiveness of vacuum for protection of pollen during transit; and (4) effectiveness of vacuum for protection of pollen at room temperature. After two years the vacuum method was better for storage than the checks but the difference wasn't of practical significance. Experiments 3 and 4 were, therefore, set up to determine if there was a real advantage in the vacuum method.

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Throughout the studies, pollen quality was evaluated by the standard North Carolina State germinability test. The pollen is germinated in a .01 percent sucrose solution for 48 hours at approximately 70°F (21°C). Percent germination is based on the number of germinated pollen grains in a 200 count. A grain is counted as germinated when the tube length is equal to or greater than the width of the pollen grain. Table 1 describes the system used to rate the pollen as to its usefulness for effecting control-pollination.

Table 1.--Rating used to determine usefulness of pollen for effecting control-pollinations

Percent Germination	Rating	Utility for Control-pollinations
35+	Excellent	Use
20 - 34	Good	Use
10 - 19	Fair	Use only if fresh pollen unavailable
1 - 9	Poor	Don't use
0	Very poor	Don't use

POLLEN EXTRACTION

The most efficient pollen extractor will be one designed to extract the pollen and dry it to a sufficiently low moisture content with the shortest exposure to room temperature. This is critical because pollen viability during storage is greatly affected by moisture content and temperature of the pollen. Pollen will quickly lose viability unless stored under near-freezing temperature. Excess moisture in pollen (especially in combination with heat) leads to molding. In fall, 1972 an extractor was constructed taking these factors into account, patterned after a smaller one constructed by the North Carolina Forest Service (Summerville and Turner, 1973). Twenty-four 12" (top diameter) gasoline funnels were modified so that the pollen from catkins placed in the funnels would collect in glass bottles attached to the bottom of the funnels. Room temperature air was blown into each bottle and escaped through the funnel, facilitating and speeding up the drying process. The 24-unit extractor was placed in a temperature-humidity control chamber, set at 70 - 80°F (21 - 27°C) and a relative humidity of about 40 percent. Equilibrium moisture content of pollen under these conditions is about 8 - 10 percent, which is sufficiently low for prevention of mold during storage. Catkins of the requested clones have been collected and shipped to North Carolina State by Cooperative members for five years now.

Many observations made during the past five years concerning the correlation between extraction and viability are:

1. Condition of catkins upon arrival has a significant effect on extraction and viability. Unripened catkins shed very little pollen, regardless of the duration of extraction. Yield of pollen from ripe, wet catkins is poor and the pollen is prone to mold. Best yields and viability of pollen are obtained from ripe, dry catkins. Best conditions for transporting

catkins to assure good yield and viability are created by collecting them when they are ripe and predrying them two to three days before shipping.

2. If mold starts in the catkins or pollen during shipment or extraction, the chances of the pollen storing well are greatly reduced.

3. The most important element in successful pollen storage is proper extraction whereby the catkins and pollen are kept dry at all times and can be moved quickly into cold storage.

Based on these observations, the following recommendations are made for collection and shipment procedures:

1. Ideally the best time to collect catkins is at the first sign of pollen shedding. However, this is a very dangerous stage as all the pollen could fly within a couple of days or within several hours if the weather conditions are right. Therefore it is desirable to collect the pollen at an earlier stage, perhaps when the bases of the catkins just start to break open or when they become yellow but produce very little liquid when squeezed between the fingers.

2. Preparation of catkins for shipping is one of the most critical aspects of pollen handling. If improperly packed, the green, moist condition of the catkins causes heat buildup and mold very quickly. Once molding has begun, the pollen is not any good. Several steps should be taken to avoid this problem.

   a. Collect catkins by clusters instead of individual catkins. This allows more aeration and less packing.

   b. If catkins are still very green, put them in paper bags (not over one-fourth to one-half full), hang them in a dry room and circulate air around them by means of a fan for a couple of days before shipping. This process will drive some of the moisture off the catkins, thereby reducing the chance of molding during shipping.

   c. When preparing for shipping, line paper bags with paper towels for absorption of excess moisture. Make only two to three narrow folds at the top of the bag and securely staple it closed. Never roll or fold the top of the bag down to the point that the catkins are tightly packed together and have no "breathing room."

   d. The paper bags should be loosely packed in a cardboard box with newspapers in between them. Holes three inches square (76 mm square) should be cut in the box for aeration.

   e. Sausage casing is less desirable for shipping catkins than paper bags. These casings are more conducive to heat and moisture buildup than are the paper bags.

POLLEN STORAGE

In establishing a research pollen bank it is necessary to use a method that will allow for successful storage of pollen for five years or longer. This option decreases the need to collect pollen each time it is needed, and it serves as insurance for gene conservation if disaster should strike the parent. In evaluating the options, it was determined that freezing and vacuum storage had been used for long-term storage of pollen (Hermann, 1969; Ching and Ching, 1964; King, 1961), and that pollen had been flame-sealed under vacuum (Jensen, 1970). Using these leads, a flame-seal vacuum device was constructed at North Carolina State based on a similar device being used at the time to store Cronartium fusiforme aeciospores. The system was put into use in spring, 1973 for storing the pollen in the pollen bank and at the same time a study was initiated to test the effectiveness of the vacuum system. The following conventional methods of pollen storage were used as checks for the vacuum storage study: (1) stored in air-tight bottles in refrigerator at 35°F (2°C); (2) cotton-stoppered bottles in refrigerator at 35°F (2°C); and (3) cotton-stoppered bottles inside desiccator (over LiCl) refrigerated at 35°F (2°C). Pollen from three clones with viability in excess of 66 percent and moisture content of 6 - 9 percent were chosen for the study. Two new methods of storage were tested. They were (1) vacuum storage under refrigeration at 35°F (2°C), and (2) vacuum storage under freezing conditions at -9°F (-20°C).

Equal amounts of pollen from each clone were placed in thirty-two 2-ml ampules (32 ampules/clone x 3 clones = 96 ampules) on April 6, 1973. The ampules were flame-sealed under a vacuum of approximately 38-mm Hg. and then placed in storage. Germination tests were made at 3, 6, 12, 18, 24, 36 and 48 months. Table 2 lists the average germination by treatment after three and four years of storage.

Table 2.--Germination (percent) of pollen stored by five different methods after three or four years of storage

Method of Storage	Percent Germination	
	Third Year	Fourth Year
Vacuum storage at 35°F (2°C)	64	29
Vacuum storage at -9°F (-20°C)	69	29
Rubber-stoppered bottle at 35°F (2°C)	15	15
Cotton-stoppered bottle at 35°F (2°C)	22	18
Cotton-stoppered bottle in desiccator at 35°F (2°C)	4	0

At the end of the third year, pollen under the vacuum had shown no noticeable decrease in germination and had stored much better than any of the checks. There was very little difference between the freezer and refrigerator treatments. Germination results from the checks were rather inconsistent and much poorer. Part of the poorer results of the checks may possibly be due to an inadvertent bias in that each check bottle was exposed to a sudden temperature change at each assessment while a new vial was available each time for the vacuum ampules.

Fourth-year results reflected an important decline in germination for every storage method. Essentially all of the check pollen was dead, with the exception of clone B which tested 46 percent germination with both the rubber-stoppered check and the cotton-stoppered check (Table 3). There was also an important decline in germination with the vacuum method except for the same clone which maintained 56 (62 and 50) percent average germination; the other clones averaged 18 (17 and 18) and 14 (10 and 18) percent. These results indicate a strong clonal variation in storage ability of pollen. Clone B stored the best in all treatments except for the desiccator treatment in which all pollens were dead after four years. This method proved the least effective of all. Average germination of the two vacuum stored treatments (freezer versus refrigerator) was the same after four years (29 percent). The 29 percent germination is very sufficient for making controlled crosses; however, the 29 percent is not actually representative but is a reflection of the unusually high value of clone B (56 percent) versus the low values of 18 percent and 14 percent for the other two clones. Germinations of the latter two clones are still acceptable for making control crosses but would be rated "fair" and "use only if fresh pollen is not available."

Table 3.--Clonal variation in germination after four years of storage by five different techniques

	Percent Germination			
Method of Storage	Clone A	Clone B	Clone C	$\bar{X}$
Vacuum storage at 2°C	17	62	10	29
Vacuum storage at -20°C	18	50	18	29
Rubber-stoppered bottle at 2°C	0	46	0	15
Cotton-stoppered bottle at 2°C	9	46	0	18
Cotton-stoppered bottle in desiccator at 2°C	0	0	0	0
Clonal Average	9	41	6	

The sudden decline between the third- and fourth-year assessments of the vacuum method was unexpected. The decline indicates one of two things: (1) vacuum storage of loblolly pine pollen under the conditions we used is only effective for three years of storage, and (2) there was a sampling error or an error in the 1977 viability tests. The study will be continued one more year and the final assessment should tell if there is validity to alternative #2. It will be remembered that separate ampules are sampled in different years, so aberrant results within one year will have no effect on those of another year.

EFFECTIVENESS OF VACUUM FOR PROTECTION OF POLLEN DURING TRANSIT

In summer, 1975 a study was conducted to determine if vacuum storage would afford good protection of pollen during transit, as suggested in the literature (Wilcox, 1966). One vacuum ampule and one screw-cap bottle (nonvacuum) containing pollen of each of three clones were sent by first-class mail from Raleigh to three cooperators, one located in Georgia, Alabama and Tennessee. The pollen

used was excess from the spring, 1975 collection and had been stored under refrigeration in air-tight bottles until time of the study, when part of them were sealed under vacuum. The pollens were packaged in polyfoam mailers (normally used for shipping blood samples) on June 30, 1975. Upon receiving the pollen, each cooperator returned them to Raleigh. The pollens arrived back in Raleigh after being in transit for a total of 9 - 10 days. Germination tests run before shipping and upon return to Raleigh are reported in Table 4.

Table 4.--Initial germination (percent) and final germination of pollen of three clones after nine to ten days in transit

Clone	Initial Percent Germ.	Final germination (%) upon arriving back in Raleigh					
		Georgia		Alabama		Tennessee	
		Vial	Ampule	Vial	Ampule	Vial	Ampule
20-506-1	47	30	52	55	61	29	34
11-61	36	0	0	0	0	0	0
8-526-4	52	34	26	63	65	36	49

Surprisingly there was little difference in germination between pollen shipped in bottles and pollen in the vacuum ampules, as both afforded good protection to the pollen. Two of the clones stored very well under both treatments, while 11-61 (initial germination of 36 percent) returned dead in all cases. Probably the 11-61 pollen was in a weakened condition at the beginning of the study, due to some detrimental environmental conditions during collection, shipping, or extraction. Possibly mold got into the pollen during prestorage treatments but was retarded by the low storage temperature, only to be reactivated again as soon as it was removed from refrigeration. In a couple of instances for the other two clones, the final germination was higher than the initial. However, this does not represent an increase in viability of the pollen but is due to experimental error in the sampling technique.

A possible explanation for the unexpected similarity between the two methods would be the effectiveness of the polyfoam containers in protecting the pollen from heat buildup. To determine if the polyfoam containers were masking differences between the two methods, another test was conducted wherein the pollen was subjected to room temperatures without use of the polyfoam containers.

EFFECTIVENESS OF VACUUM FOR PROTECTION OF POLLEN AT ROOM TEMPERATURE

This test was designed to determine the effects of continuous room temperature storage on the germinability of pollen stored in screw-cap bottles and in the vacuum ampules.

Pollen from three clones was chosen for this experiment, based on germination tests conducted a few days prior to initiation of the study. The pollen used was excess from the spring, 1975 collection and had been stored in air-tight bottles under refrigeration until the time of the study. Average pollen germination for the three clones at the start of the study was 63 percent.

The experiment ran for eight weeks. Eight vacuum-sealed ampules and eight air-tight (nonvacuum) bottles of pollen from each clone were prepared on July 22, 1975 and were placed in a room where the temperature averaged 80°F (27°C). One ampule and one bottle of pollen from each clone were tested for germination each week during the eight-week period. Germination results are recorded in Table 5.

Table 5.--Average[1/] weekly germination of pollen stored in air-tight bottles and vacuum-sealed ampules under room temperature of 80°F (27°C) for eight weeks

Assessment Time	Air-tight Bottle	Vacuum Ampule
First week (July 29)	54	60
Second week (August 5)	21	40
Third week (August 12)	35	53
Fourth week (August 19)	8	27
Fifth week (August 26)	0	45
Sixth week (September 2)	1	10
Seventh week (September 9)	0	12
Eighth week (September 16)	0	18

[1/] Average of the three clones

The vacuum-sealed pollen rated higher in germination each week. After the fifth week, pollen stored in the bottles was essentially dead. The vacuum-sealed pollen dropped noticeably in germination between the fifth and sixth week but still rated fair through the end of the eighth week, at which time it tested at 18 percent germinability, which is still high enough to consummate control-pollinations. Germination percent for the pollen under vacuum actually showed an increase from the sixth week to the eighth (10, 12, 18); however, as mentioned earlier, this type variation is due to experimental error in the sampling technique. These results indicate that under extreme temperature conditions the vacuum ampules definitely afford the best protection.

SUMMARY AND DISCUSSION

During the past five years, experience in pollen extraction and storage has taught us that the most critical factor in maintaining viable pollen is to control its moisture content. Pollen stored at a 10 percent moisture content maintained sufficient viability for up to three years for most of the methods tested. Pollen stored at a moisture content greater than 10 percent has the propensity to mold. Mold often occurs in pollen during the process of moisture content reduction brought about by a desiccant such as lithium chloride. To avoid the problems, the recommended procedure is to reduce the moisture content of the pollen during the extraction process. It is preferable to keep the pollen in the extractory a few days longer than is normal, to assure the proper moisture content level of about 10 percent.

Results from these studies indicate that the vacuum method of pollen storage has definite advantages over the nonvacuum methods. The pollen under vacuum stored more consistently for three years than the check pollen. Part of the difference in germination between the vacuum-stored pollen and the checks could be due to an experimental bias against the checks. Further studies will be carried out to determine if in actuality this bias exists. If the pollen can be stored in air-tight bottles for three years with good germination, the vacuum method may not be needed. The ampules offer no advantage in shipping as the polyfoam packers afford good protection to the pollen, regardless of how it is stored. In the meantime the North Carolina State Cooperative will continue to use the vacuum method to assure the best pollen quality possible, as this method has produced the most consistent results.

It was hoped that vacuum storage would be effective for long-term storage of pollen (3+ years), but the fourth-year results tend to dispel that hypothesis. The fifth-year (final) assessment should tell if an error was committed in the 1977 assessment. If germination is low next year, then the vacuum storage effectiveness will be about three years. If germinations are higher next year, then perhaps there was some error in the test method at the fourth year; and perhaps the vacuum method will be effective for more than three years. Whatever the final results indicate, it would be difficult to justify the expensive vacuum method for normal pollen storage by an individual industry. It would be more readily justified for a research pollen bank like the one at North Carolina State University.

LITERATURE CITED

Ching, Te May and Kim K. Ching. 1964. Freeze-drying pine pollen, Plant Physiology 39(5):705-709.

Hermann, S. 1969. Practical method to conserve pollen of forest trees. Voluntary paper presented at the Second World Consultation on Forest Tree Breeding, Washington, D. C. August 7 - 16. FO-FTB-69-11/10. 7 pp.

Jensen, C. J. 1970. II. Some factors influencing survival of pollen on storage procedures. FAO-IUFRO, Sec. 22, Working Group Meeting on the Sexual Reproduction of Forest Trees, Varparanta, Finland.

King, J. R. 1961. The freeze-drying of pollens. Econ. Bot. 15(1):91-98. Forestry Abstracts 22(4):518.

Summerville, K. O. and S. F. Turner. 1973. New equipment improves pollen viability. Tree Planter's Notes 24(1):3-6.

Wilcox, J. R. 1966. Vacuum storage of yellow-poplar pollen. Joint Proceedings, Second Genetics Workshop of SAF, and Seventh Lake States Forest Tree Improvement Conference. USFS Res. Pap. NC-6, North Central For. Expt. Sta.

POLLEN QUANTITY AFFECTS CONE AND SEED YIELDS IN CONTROLLED SLASH PINE POLLINATIONS

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Abstract.--Controlled pollination treatments ranging from 0-32 puffs of pollen per bag were tested on slash pine in the Georgia Forestry Commission's Arrowhead Seed Orchard, Pulaski County, Georgia. Cone survival, cone size, seed yield, and seed efficiency increased with increased quantities of pollen. Filled seed yields ranged from 0 in the unpollinated cones to 100 seeds per cone in the 32-puff treatment. The efficiency of production of viable seeds was five times greater for the 32-puff treatment than the 2-puff treatment. Applying large quantities of pollen would enable the seed orchard manager to pollinate fewer bags, yet still provide adequate amounts of seed for progeny testing or advanced breeding.

KEYWORDS: Seed orchard, seed efficiency, seed production, *Pinus elliottii*.

Controlled pollination is required in progeny tests, advanced breeding programs, and interspecific hybridization of southern pines. This work is time-consuming and expensive and should be designed to produce seeds as efficiently as possible. Seed yields from controlled pollinations, however, have been much lower than the seed potential of the cones (DeBarr and others 1975; Snyder and Squillace 1966). One possible cause of low seed yields is application of too little pollen. This study evaluated the effect of the pollen quantity on the seed and cone yields from controlled pollinations of slash pine.

METHODS

Five slash pine trees, each representing a different clone, in the Georgia Forestry Commission's Arrowhead Seed Orchard, Pulaski County, Georgia, were selected as study trees. On each tree, 48 flower bearing branch tips were tagged and 6 branches were randomly assigned to each of 8 pollination treatments.

Treatments 1 through 6 were controlled pollinations with 0, 2, 4, 8, 16, and 32 puffs[2/] of pollen applied to each bag with a standard syringe and bulb pollinator (Mergen and others 1955). One puff of the syringe was equal to roughly 0.06 ml of pollen or approximately 6×10^5 individual pollen grains.[3/] The pollen

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2/ A puff of the syringe pollinator was generated by expelling the air from the bulb.

3/ Personal communication, Fred Matthews, USFS, Athens, Georgia.

supply was a fresh, five-tree polymix of unrelated clones from the Arrowhead Seed Orchard. Pollen was applied at only one time to each bag. Treatments 7 and 8 were wind pollinations and did not include any supplemental pollen. All study trees were sprayed with Guthion® insecticide at monthly intervals from April-October of 1975 and 1976. Furadan® 10G was applied to each study tree in February 1976, at a rate of eight ounces per inch of tree diameter. In addition, fiberglass screen cages (Bramlett and others 1977b) were installed on the conelets in treatments 1 through 7 in 1975. The new vegetative growth was released from the distal end of screens in spring of 1976 and the screens maintained until cone maturity.

All mature cones were collected in September 1976. From each study tree, five cones in each treatment were randomly selected for analysis. Sample cones were air dryed until the scales began to separate and then dried in a forced-draft oven at 40°C for 24 hours. All loose seeds were extracted from the cone. Then all cone scales were removed and examined to determine the numbers of total scales, fertile scales (seed potential), aborted ovules, and unextracted seeds. All developed seeds from the cone were radiographed and classified as filled, empty, malformed, fungus damaged, or incomplete gametophyte by the method of Bramlett and others (1977a).

Seed germination was measured after 30 days in sand flats on greenhouse benches. Analyses of variance were used to evaluate the effects of pollen treatments on the cone, seed, and germination performance of the study trees.

RESULTS AND DISCUSSION

Cone Survival

Unpollinated flowers frequently aborted before cone maturity and had an average of 28 percent survival on the five study trees. Flowers pollinated with 2 puffs of pollen averaged 55 percent survival. Treatments with 4, 8, 16, and 32 puffs had an average of 75, 77, 75, and 70 percent survival respectively, but the mean survival values for all pollination treatments (2-32 puffs) were not significantly different. Wind-pollinated flowers averaged 71 percent survival with screens and 70 percent without screens.

Some cone losses were caused by mechanical (broken branches, etc.), insect, and fungus damage. Dead cones with no external symptoms of damage were classed as aborted. Cones not present at the periodic counts were recorded as missing. When the survival percentage of cones was adjusted to consider only mortality in the aborted and missing classes, cone survival averaged 87 percent in the controlled pollinated cones; 88 percent in wind-pollinated cones, and 36 percent in unpollinated cones.

Cone Morphology

The few unpollinated cones that survived were much smaller than the pollinated cones. Both cone length and width increased with increasing quantities of pollen applied (table 1). From these data it appears that the developing seed influence, to some degree, the scale development and size of the mature cone.

Table 1.--Average cone size, number of scales, and seed potential from pollination treatments in five slash pine study trees

Pollination treatment (puffs)	Cone length 1/	Cone width 1/	Fertile scales 2/	Total scales 2/	Seed potential 2/
	--------mm--------		----------number----------		
0	98	33	86	158	171
2	111 c	40 c	86	159	171
4	114 bc	41 c	91	166	183
8	113 bc	42 c	86	162	172
16	120 bc	46 b	89	164	177
32	123 b	47 b	90	167	179
Wind 3/	115 bc	46 b	90	164	180
Wind 3/	133 a	51 a	89	161	179

1/ Values not followed by the same letter are significantly different at the 0.05 level of probability by Duncan's New Multiple Range Test. Treatment 1 was not included in analysis of variance because of missing values due to conelet abortion.
2/ No significant differences between treatments.
3/ Cone and conelets not protected with screen cages.

Caging significantly reduced the size of wind-pollinated cones (treatment 7 vs treatment 8). Apparently the cage reduced light intensity and thus reduced the quantity of photosynthate supplied to the developing cone. No other measured morphological trait (including the number of fertile scales, total scales, and the seed potential) was influenced by the pollination treatment (table 1).

Seed Yield

The total number of seed as well as the filled seed yield from mature cones varied directly with the quantity of pollen applied to the flowers (table 2). As expected, unpollinated cones yielded no seeds. Total seeds/cone increased from 57 with 2 puffs of pollen to 138 with 32 puffs. Wind-pollinated cones averaged 157 seeds per cone with screens and 129 seeds per cone without screens.

As pollen quantity increased from 2 to 32 puffs, the yield increased from 29 to 100 filled seeds per cone. Wind-pollinated cones averaged 120 filled seeds per cone with screen cages and 114 filled seeds per cone without screen cages.

Table 2.--Average seed yields and seed losses from pollination treatments in slash pine

Pollination treatment (puffs)	Aborted ovules		Developed seed					
			Seed losses					
	First year [1]	Second year [2]	Incomp. [2]	Malform. [2]	Fungus [1]	Empty [1]	Filled [1]	Total [1]
	number							
0	171	0	0	0	0	0	0	0
2	111 ab	3	6	0	3 abc	21 abc	29 c	57 c
4	117 a	1	4	0	2 bc	20 bc	38 c	64 c
8	90 b	3	3	0	6 ab	24 abc	45 c	79 c
16	60 c	5	4	0	8 a	24 abc	77 b	113 b
32	39 cd	3	4	0	7 ab	27 ab	100 a	138 ab
Wind [3]	19 d	4	4	0	2 bc	32 a	120 a	157 a
Wind [3]	46 c	4	1	0	0 c	14 c	114 a	129 b

1/ Values not followed by the same letter are significantly different at the 0.05 level of probability by Duncan's New Multiple Range Test. Treatment 1 was not included in analysis of variance because of missing values due to conelet abortion.
2/ No significant differences between treatments.
3/ Cones and conelets not protected with screen cages.

Seed Losses

Since the only product of value to the tree improvement program is filled seeds, all other classes of ovules and seeds are considered as seed losses. These losses include ovules that aborted before seedcoat development.

In contrast to the seed yields, the seed lost as first-year aborted ovules, i.e., ovules aborting during conelet stage (Bramlett 1974), decreased as the quantity of applied pollen increased (table 2). All ovules in unpollinated cones (171 per cone) were classified as first-year aborted. In the 32-puff treatment an average of 39 ovules per cone were in this category. These results confirm a previous report that lack of viable pollen causes the ovule to abort during the first year of development (McWilliam 1959). The larger number of aborted ovules in the unscreened, wind-pollinated cones indicates that some ovules were lost to seedbugs. These observations substantiate the report of DeBarr and others (1975) that both insects and lack of pollen cause abortion of ovules in slash pine seed orchards.

Very few second-year aborted ovules were observed in the study and pollination treatments were not significantly different. None of the second-year ovules appeared to abort from insect damage.

Developed seed losses were classed as incomplete gametophytes, malformed, fungus, or empty seed. No insect damage was observed on the seed radiographs.

Seeds with incomplete gametophytes were infrequent in all treatments and although some malformed seeds were observed in all pollination treatments, all treatments averaged less than one malformed seed per cone.

Some seeds were lost to fungi in all treatments except the wind pollination treatments with no screen cage. These seed losses could be related to the nutritional status of the cone or to the increased fungal damage in the constrictive cage. Caged branches frequently contained dead or damaged needles and fungal mycelium on the cone scales.

The empty seed class included the typical "pops" in which only a remanent of the embryo and gametophyte are present. These seed losses increased slightly in number as the pollen quantity increased, but the percentage of empty seeds decreased because fewer total seeds were produced in cones that received less pollen. The number of empty seeds, however, was greater in the wind-pollinated cones with screen cages than in cones without screens.

Seed Production Efficiency

The efficiency of seed production was rated for each pollination treatment by comparing actual with potential yields of cones, seeds, and seedlings. Thus, cone efficiency (CE) is the ratio of healthy mature cones to pollinated flowers. Only unpollinated flowers had noticeably reduced cone efficiency, although the 2-puff pollination did have a lower mean cone efficiency than other pollination treatments (table 3).

Seed efficiency (SE), the ratio of filled seed to the seed potential for each cone, is the most important measure of pollination effectiveness. It compares the number of seeds the normal reproductive system produces with the number of functional ovules in the cone.

Extraction efficiency (EE) measures the success of seed extraction from mature cones. The degree of cone opening was the most important factor in seed extraction. The slash pine cones evaluated in this study generally opened well and extraction efficiency averaged 90 percent. Some cones with fungal mycelium on the scales did not open as well as the cones with no fungi. In general, there was little or no effect of the pollen treatments on seed extraction.

Germination efficiency (GE) is the ratio of the number of germinated seed to number of filled seed produced by the cone. The germination percentage was generally less for caged cones than for wind-pollinated, uncaged cones. Apparently, the reduced germination was related to the higher frequency of fungus damage and incomplete gametophyte development in the caged cones.

Seed Orchard to Nursery Efficiency

The seed orchard to nursery efficiency (SO-NE) was calculated as a product of cone efficiency, seed efficiency, extraction efficiency, and germination efficiency:

$$SO\text{-}NE = CE \times SE \times EE \times GE.$$

The SO-NE considers the loss ratios of flowers to cones, seed potential to filled seed, extraction of seed, and the germination process. This value was then a ratio of the number of seedlings produced from the cones collected from a given number of pollinated flowers. For example, in the 2-puff treatment, the overall cone efficiency was 55 percent (CE = 0.55) and the seed efficiency was 16 percent (SE = 0.16). The extraction efficiency was 82 percent (EE = 0.82) and the percent germination of filled seeds was 70 percent (GE = 0.70). Thus, the SO-NE would equal 0.55 x 0.16 x 0.82 x 0.70 = 0.05. In comparison, in the 32-puff treatment, the following values were observed: CE = 0.70; SE = 0.56; EE = 0.91; and GE = 0.73. Thus, SO-NE = 0.70 x 0.56 x 0.91 x 0.73 = 0.26 or 5 times as great as the 2-puff treatment. The SO-NE for wind pollinations was 0.32 caged cones and 0.36 for uncaged cones.

Table 3.--Cone, seed and seedling efficiency from slash pine cones pollinated with varying quantities of pollen

Pollination treatment (puffs)	Cone efficiency 1/ 6/	Seed efficiency 2/ 6/ 7/	Extraction efficiency 3/ 6/ 7/	Germination efficiency 4/ 8/	Seed orchard-nursery efficiency 5/
	----------	----------	Percent ----------	----------	----------
0	28 a	0	0	0	0
2	55 b	16 c	82 bc	70	5
4	75 b	21 c	84 abc	62	8
8	77 b	27 c	77 c	64	10
16	75 b	44 b	93 ab	65	20
32	70 b	56 a	91 ab	73	26
Wind 9/	71 b	67 a	95 a	70	32
Wind 9/	70 b	63 a	96 a	84	36

1/ Cone efficiency (CE) = Pollinated flowers/mature cones.
2/ Seed efficiency (SE) = Filled seed/seed potential.
3/ Extraction efficiency (EE) = Extracted developed seed/total developed seed.
4/ Germination efficiency (GE) = Germinated seed/filled seed.
5/ Seed orchard-nursery efficiency (SO-NE) = CE x SE x EE x GE.
6/ Values not followed by the same letter are significantly different at the 0.05 level of probability by Duncan's New Multiple Range Test.
7/ Treatment 1 was not included in analysis of variance because of missing values due to conelet abortion.
8/ No significant differences between treatments.
9/ Cones and conelets not protected with screen cages.

CONCLUSIONS

Seed and seedling yields were greatly increased in controlled pollinations of slash pine by applying large volumes of pollen (32 puffs) into each pollination bag. The overall effect of the higher density of pollen was an increased efficiency of the seed production process. On this basis, it appears that a given number of seedlings can be produced from fewer bags by increasing the amount of pollen applied to each bag. These procedures, however, would require larger quantities of pollen to be collected, processed, and stored.

The efficiency statements indicated the key stages in which seed losses were occurring. In this study, treatment effects were most important in influencing the seed yield per cone. The seed efficiency of the highest level of controlled pollination, however, did not equal the wind-pollinated control cones. It is possible that even higher yields of seed could be obtained by pollinating each bag two or more times.

Insect protection is a necessity for the success of controlled pollinations in most seed orchards. In this study, the screen cages reduced the number of first-year aborted ovules but also appeared to cause increased fungus damage, poorer gametophyte development, and poorer seed germination in the mature cones. Systemic insecticides are preferable for insect control after controlled pollinations.

LITERATURE CITED

Bramlett, D. L., E. W. Belcher, Jr., G. L. DeBarr, G. D. Hertel, R. Karrfalt, C. W. Lantz, T. Miller, K. D. Ware, and H. O. Yates, III. 1977a. Cone analysis of southern pines. Gen. Tech. Rep., USDA, For. Serv. (In press).

Bramlett, David L., William G. Lewis, and Gary L. DeBarr. 1977b. Screen cages to protect pine cones from seedbugs. Tree Planters' Notes (In press).

Bramlett, David L. 1974. Seasonal development and loss of Virginia pine ovules and seed. Seed Sci. and Technol. 2: 285-292.

DeBarr, G. L., D. L. Bramlett, and A. E. Squillace. 1975. Impact of seed insects on control-pollinated slash pine cones. Thirteenth South. For. Tree Improv. Conf. Proc. 1975: 177-181.

McWilliam, J. R. 1959. Interspecific incompatibility in Pinus. Am. J. Bot. 46: 425-433.

Mergen, Francois, Harry Rossoll, and Kenneth B. Pomeroy. 1955. How to control the pollination of slash and longleaf pine. USDA, For. Serv. Res. Pap. SE-58, 14 p. Southeast. For. Exp. Stn., Asheville, N. C.

Snyder, E. B., and A. E. Squillace. 1966. Cone and seed yields from controlled breeding of southern pines. USDA, For. Serv. Res. Pap. SO-22, 7 p. South. For. Exp. Stn., New Orleans, La.

EARLY TESTING OF LOBLOLLY PINE

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Abstract: Cones were collected from individual trees in natural stands of loblolly pine (Pinus taeda L.) in southeast Texas. Seed from each tree were evaluated for characteristics such as size, weight, percent filled seed, germination time and stratification requirements. The percent filled seed, mold on the seed during germination, stratification requirements and growth in the nursery and greenhouse were used as criteria in a step-wise screening procedure. The selected families and controls were outplanted for future evaluations to determine the effectiveness of the screening procedure. Early results indicate that information gained from seed, germination times, and early growth, can be used to increase the proportion of fast growing families in the selected group.

Additional keywords: Pinus taeda, early testing, seed germination, early growth, stratification.

Progeny testing of superior loblolly pine phenotypes is necessary before the genetic worth of the individual selection can be ascertained. This is costly not only because of the expense of progeny testing, but also because of the time lost waiting for progeny test results to become available. Because of this time lag, several workers have attempted to find correlations between early growth habits and mature field performance. Two such studies by Zobel (1953) on outstanding seedlings selected in the nursery bed and Brown (1959) on outstanding loblolly and shortleaf from seed production area trees were early approaches to this problem in southern pines.

Working with cotton, Bird and Presley (1965) and Bird (1972) developed a method for early selection of cotton for disease resistance, improvements in earliness and yield by selecting for germination at reduced temperature and absence of mold on the seed.

For loblolly pine the situation is more complicated than for cotton, since loblolly pine has a stratification requirement. Therefore a preliminary study was carried out using 9 known fast growing and 5 known slow growing families, comparing their germination without stratification, and their germination at 13°C after stratification.

This study showed that of the slow growing families more than twice as many seedlings germinated without stratification than of the fast growing families. Mold growth was also somewhat more serious on the slow growing families. There was no difference in germination at 13°C. On the basis of these results it was decided to go ahead with a larger study to determine if

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it is possible to use stratification requirements as a means of rapidly screening a large number of families. Even a modest gain would be worthwhile since it could be done very rapidly and inexpensively.

A number of additional selection criteria were added to reduce the number of families finally outplanted for testing. At each step of the screening procedure bulk lots were made up for field planting to determine the effect of that particular step.

A second major consideration in establishing this study was the need for preserving a portion of the existing gene pool in mature loblolly pine stands. As native stands are harvested the gene pool is seriously depleted and trees which could possibly be used in future breeding programs are lost.

METHODS

Cone Collection

In 1973 cone collections were made in good natural stands of loblolly pine as they were being harvested for sawlogs. Most of these stands were being clearcut. Five healthy cones were collected from each of several good trees after felling. If possible the best trees in good stands were sampled. Approximately 100 trees per stand were sampled in 10 widely scattered stands. A total of 1000 loblolly pines were sampled.

Cone and Seed Handling

Seed were extracted from the cones as soon as they opened. Hand extraction was used to get as many seed as possible from the cones short of cone destruction. Each seedlot was floated in distilled water to separate empty seed from filled seed. An electronic seed counter was used to count all seedlots for both empty and filled seed. Ten seed from each seedlot were picked to form the baseline check seedlot.

At this step in the procedure about half of the seedlots were screened from the test by eliminating them if the number of filled seed per cone was below average. This was done separately for each collection area. The remaining seedlots were weighed and stored at 1-2°C until further testing started. Five seed from each of the selected seedlots were composited to form the bulk seedlot used for evaluating the effect of screening for above average number of seeds per cone.

Germination and Mold

Germination without stratification studies were conducted on all seedlots remaining after screening for filled seed per cone. For germination tests a 1.5% water agar medium in sterilized petri dishes was used. Forty seed from each selected seedlot were placed in petri dishes and placed in continuous incandescent light at 20-21°C. Germination counts were initially made at 12 and 15 days. This was later changed to 15 days after enough data was accumulated to indicate this interval gave adequate information for measuring germination and mold.

Germination was counted if any portion of the root was visible outside the seed coat. Seed was counted as moldy if any fungus was present on the seed

whether a known pathogen or not. Among the fungi identified on the seed were: Penicillium sp., Rhizopus sp., Alternaria sp., Aureobasidium sp., Botrytis sp., and Fusarium sp.

The seedlots from each collection site with the least number of seed germinated and the least susceptibility to mold were selected for further testing. Each collection area was treated separately and a standard for mold infection as well as a separate standard for nonstratified germination was developed for each seed collection area. In this second step of the screening procedure approximately 50 percent of all remaining seedlots were selected for further testing. Five seeds of each selected seedlot were composited to form a bulk seedlot to check the effect of this step in the screening process.

Early Growth

Seed from all selected families was stratified at 2-4°C for 30 days. After stratification seed from each family was planted in the greenhouse and at Indian Mound Nursery. The greenhouse seedlings were grown in coarse sand in the greenhouse benches.

Greenhouse and nursery plantings were randomized and replicated. Greenhouse seedlings were subirrigated with a nutrient solution. Damping off was controlled with weekly Captan spray for the first two months. The nursery portion of the test received exactly the same treatments as regular progeny tests. Monthly height measurements were made from July through November and diameter measurements were made in December on the five tallest seedlings in each family of greenhouse-grown seedlings. Nursery height measurements were made in October.

Six additional traits were used to select the final families for field planting in addition to the various seedlots established to check on the effect of the steps in the screening procedure. The variables used were final height in the greenhouse, final diameter in the greenhouse (average of the five tallest trees in each plot) and final height in the nursery.

Since these measurements were correlated with seed size (table 1) and in case of the greenhouse study with position in the bench, values adjusted for these variables were also calculated as deviations from the regression line. The 14 or 15 highest ranking families in each of the categories were finally selected for field planting, if a sufficient number of seedlings were available.

Table 1.--Correlation between seed weight and growth characteristics

Traits	Correlation Coefficient
Seed weight-diameter of greenhouse grown seedlings	.48
-height of greenhouse grown seedlings	.37
-height of nursery grown seedlings	.51

Because of the environmental differences between greenhouse and nursery, only seedlings grown at Indian Mound Nursery were outplanted. Six replications of 8 tree row plots were planted at the Spurger progeny test area.

RESULTS AND DISCUSSION

Field results will not be available until the fall of 1979. The results summarized in tables 2, 3, and 4 are therefore limited to laboratory, greenhouse and nursery data.

Table 2.--Ranking of families according to six different traits

Family	Nursery Height	Adjusted Nursery Height	Greenhouse Height	Adjusted Greenhouse Height	Greenhouse Diameter	Adjusted Greenhouse Diameter	Number of Seedlings
A-2	4	6					84
A-31		13					79
A-53	14	12	12	14		6	66
A-57			13		4		53
A-76	2		2		5		72
A-82	1	2	4		9		90
A-85			1	1	2	10	63
D-113	13	5					72
D-125			8	4			83
D-154	6	4					75
D-159	10						79
E-2					6	1	56
E-95						14	89
F-48	11		3	9	1		62
L-1					10	7	74
L-34		8		8			62
L-77		14	14	10			78
L-79			11	11			73
L-88			7	7			53
L-89			10	6			51
M-128	5	1					89
N-8					14	9	100
N-51			5	2	7	4	54
S-34	3	3					75
S-65	7						72
S-68		7					65
S-74	8	9					75
Z-106	9						92

Table 3.--Seed weight, germination, and growth of families selected for field planting

Parent Tree	Weight/ 100 seed (g)	Percent Germination (Unstratified)	Percent Mold Infection	Height Growth (cm)				Diameter Growth (cm)	
				Nursery		Greenhouse		Greenhouse	
				Actual	Adjusted	Actual	Adjusted	Actual	Adjusted
A-2	2.96	0	0	25.7	4.79	19.5	-1.30	.361	-.027
A-31	1.93	5.0	0	20.6	3.73	21.1	3.42	.345	.013
A-53	2.44	2.5	0	23.0	4.19	24.0	4.28	.416	.061
A-57	2.73	5.0	0	18.7	-1.25	23.8	1.63	.452	.047
A-76	3.50	5.0	0	26.2	3.16	28.0	3.64	.447	-.014
A-82	3.38	5.0	0	28.4	5.85	27.4	3.79	.434	-.012
A-85	2.58	0	0	18.9	-0.51	28.2	7.07	.457	.052
D-113	2.19	7.5	15.0	23.0	5.19	20.7	2.72	.333	-.020
D-125	2.06	7.5	10.0	20.7	3.36	25.7	6.01	.381	.019
D-154	2.46	0	12.5	24.2	5.23	19.3	-0.21	.350	-.036
D-159	2.84	5.0	10.0	23.4	2.97	19.0	-0.54	.343	-.035
E-2	2.58	0	7.5	20.0	0.60	23.4	2.86	.444	.073
E-95	2.21	5.0	2.5	16.7	-1.23	18.3	-0.08	.411	.049
F-48	3.03	0	10.0	23.4	2.18	27.9	4.88	.462	.041
L-1	2.61	0	0	17.2	-2.35	21.8	2.58	.432	.058
L-34	1.80	2.5	0	20.8	4.54	21.3	4.93	.328	.003
L-77	2.13	5.0	0	21.3	3.72	23.7	4.66	.358	-.005
L-79	2.63	5.0	0	18.1	-1.53	24.0	4.61	.381	.018
L-88	2.48	5.0	0	16.5	-2.53	26.0	5.42	.389	.014
L-89	2.53	5.0	0	17.8	-1.39	24.3	5.62	.371	.022
M-128	2.59	0	2.5	25.4	5.97	21.3	1.21	.391	.014
N-8	2.47	5.0	0	19.7	0.78	19.0	0.20	.422	.054
N-51	2.58	0	5.0	19.3	-0.08	26.5	6.51	.444	.066
S-34	2.83	2.5	0	25.8	5.41	20.5	0.52	.325	-.046
S-65	2.78	15.0	5.0	23.8	3.66	18.6	-2.15	.320	-.060
S-68	2.08	15.0	0	22.2	4.74	16.2	-3.13	.333	-.021
S-74	2.54	2.5	2.5	23.8	4.53	20.4	1.44	.381	.009
Z-106	3.32	0	2.5	23.7	1.35	14.6	-5.24	.328	.051
Average	2.58	3.9	3.0	21.7	2.32	22.3	2.33	.387	.014

Table 4.--Effect of the different steps of the screening process on seed and seedling characteristics

	Nursery Height	Adjusted Nursery Height	No. Filled Seeds/Cone	Moldy Seeds, %	Germination without Stratification,%
Base population	20.2	1.90	71.54	NA	NA
Families with above average filled seed/cone	20.1	1.64	95.66	10.2	9.4
Families with lower mold and prestratification germination	18.4	.05	95.00	3.2	2.7
Families selected for field planting	21.7	2.32	93.09	3.1	4.1

Seed

As would be expected there was a considerable difference between collection sites in the average number of seed per cone, average number of filled seed, average weight per 100 seed and number of seed per pound. However, there were no readily discernable geographic trends evident in these measurements. For most collection areas the variation within the area was generally as great as variation between areas. This part of the study will be summarized and published in a separate article.

Molds

Percent moldy seed ranged from 0 to 75 with an average of 10.2. Percent mold of the seedlots passing the screening process ranged from 0 to 17.5 with an average of 3.0.

Germination without Stratification

Germination without stratification ranged from 0 to 67.5 percent with an average of 9.4. The seedlots passing the screening process had an average germination without stratification of 3.9 percent ranging from 0 to 17.5 for individual seedlots.

Growth in the Greenhouse and Nursery

Average height of the seedlings grown in the greenhouse was 19.2 cm, with the family means ranging from 12.8 cm to 28.3 cm. The average of the group selected for height growth in the greenhouse was 25.8 cm.

The height values adjusted for seed size and edge effects in the greenhouse benches averaged 0, since they were deviations from regression, and ranged from -5.2 to +7.1 cm. The average of the selected group was 5.4 cm.

Average height of the seedlings in the nursery was 18.3 cm. Family means ranged from 10.2 cm to 28.4 cm. The mean of the group selected for height growth in the nursery was 24.6 cm.

The family averages for nursery height adjusted for seed size, again expressed as deviations from the regression, ranged from -5.5 cm to +6.0 cm, averaging 0. The average of the group selected for adjusted nursery height was 4.8 cm.

The families averaged .361 cm in diameter (measured on the 5 tallest trees in each plot in the greenhouse), ranging from .264 cm to .462 cm. The average of the group selected for diameter was .444 cm.

The diameter adjusted for seed size and edge effects ranged from -.079 cm to +.074 cm, averaging again 0. The average of the group selected for adjusted diameter was .058 cm.

Discussion

The effect of the various steps in the screening process are summarized in table 4. Selection for number of filled seed per cone had no effect on height growth in the nursery. On the other hand selection for freedom of mold and low germination without stratification resulted in somewhat decreased growth in the nursery, although the difference was not statistically significant. All these families have been field planted and are now in their third growing season. Field observations show that some of the outstanding families are still maintaining their initial superiority. Since experience with progeny tests has shown that early field measurements do not correlate well with measurements taken at 20-years and later, the first measurements on these field plantings will be taken in 1979, when they have completed their fifth growing season in the field. Although even those measurements will be preliminary, they should give a good indication if any of the steps in the screening procedure have been effective.

LITERATURE CITED

Bird, L.S. 1972. Interrelationships of resistance and escape from multi-disease and other adversities. Beltwide Cot. Prod. Res. Conf., Cot. Dis. Council. 23:92-97.

Bird, L.S. and Presley, J.T. 1965. Cotton seedlings disease escape-resistance to seed deterioration and low temperature germination and growth. Proc. Cot. Dis. Council. 25:88-89.

Brown, C.L. 1959. Seventh Progress Report of Cooperative Forest Tree Improvement Program of the Texas Forest Service, Circular 62. pp. 11-12.

Zobel, B.J. 1953. First Progress Report, Forest Tree Improvement Program of the Texas Forest Service, Circular 35. pp. 12-13.

PATTERNS OF FUSIFORM RUST INCREASE AND THEIR IMPLICATIONS FOR SELECTION AND BREEDING

M. M. Griggs and R. J. Dinus[1/]

Abstract.--When increase of fusiform rust was observed for 12 years in two progeny tests of six open-pollinated slash pine families, family rankings did not change appreciably from the 3rd year after planting. Noncumulative estimates of rust incidence reflected family rankings as accurately as cumulative estimates. Noncumulative and, especially, cumulative estimates were highly correlated with results from artificial inoculations. Cumulative records, however, are necessary for accurate second generation selection. Without individual tree histories, substantial numbers of selections would be escapes or previously galled trees that became rust free through natural pruning. Such errors are quite frequent in progeny tests with light to moderate infection levels. The course of rust-associated mortality paralleled that for total infection and stem infection. Infected trees in one family were better able to withstand and survive infection than those in a similarly infected family.

Additional keywords: Cronartium fusiforme, Pinus elliottii var. elliottii, P. taeda, tolerance, epidemiology.

INTRODUCTION

Fusiform rust (Cronartium fusiforme Hedgc. & Hunt ex Cumm.) limits efficient management of slash (Pinus elliottii var. elliottii Engelm.) and loblolly (P. taeda L.) pines over much of their commercial range. Genetic variation in resistance is substantial in both pines (Rockwood and Goddard 1973, Stonecypher et al. 1973) and resistance breeding is widely used for reducing losses.

Despite much progress, commercial breeding programs still rely on few resistant parent trees, and much remains unknown about different types of resistance and their relative frequencies. Pathogenic variability in C. fusiforme is considerable, and forms are capable of negating resistance in some slash pines (Dinus et al. 1975). Increased emphasis must therefore be placed on acquiring more resistant parents and determining the nature of their resistance so that future planting stock contains a variety of resistant genes.

This report describes the course of rust infection in two open-pollinated slash pine progeny tests. Patterns of rust increase over the 12 years after

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planting illustrate how much infection occurred in each of six families, when and how rapidly infections accumulated, and how each family responded. Results suggest several types of resistance are available even in this limited sample of slash pine. Implications concerning selection for second generation breeding and relationships between artificial inoculation results and field performance are also discussed.

MATERIALS AND METHODS

Open-pollinated progeny tests of six south Mississippi slash pines were established in 1963 near Gulfport, Mississippi (Location 1), and in 1964 near Bogalusa, Louisiana (Location 2). All six families were planted at Location 1 in a randomized block design. Each family is represented by a 30-tree plot in each of seven blocks. At Location 2, five families, excluding Family 4, are represented by 30-tree plots in each of five blocks. Early field performance and its relationship to artificial inoculation results were described earlier (Dinus 1969).

Galled trees, trees with stem galls, and trees killed by rust at Location 1 were counted annually the first 6 years after planting, and at the end of the 9th, 10th, and 12th growing seasons. The same counts were taken annually the first 4 years at Location 2, and at the end of the 6th, 10th, and 12th growing seasons. Data were summarized to give three cumulative estimates and one noncumulative estimate of rust incidence and severity.

Cumulative estimates included: (1) cumulative percent galled (CPG)--total trees having had at least one gall as a percentage of trees living plus those killed by rust; (2) cumulative percent stem galled (CPSG)--total trees having had at least one stem gall as a percentage of trees living plus those killed by rust; and (3) cumulative percent rust-associated mortality (CPRAM)--trees killed by rust as a percentage of trees living plus those killed by rust. In developing cumulative estimates, trees dying of causes other than rust were removed from the data during and after the year in which they died. Such mortality was greatest in the first 2 years after planting, was not correlated to relative family resistance (Dinus 1969), and is not expected to influence outcome of the analyses.

The noncumulative estimate was percent galled (NCPG)--the number of trees having at least one gall on a given observation date as a percentage of trees living at that time. NCPG is a point-estimate variable and has been used frequently to evaluate rust incidence in progeny tests, provenance trials, and commercial plantations (Wells and Wakeley 1966, Rockwood and Goddard 1973, Stonecypher et al. 1973).

Percentages for each variable were calculated on a plot basis and subjected to analyses of variance for the randomized block design. Analyses combining locations were not attempted because location effects would have been confounded with different planting dates and numbers of families. Family differences at various times were compared by Duncan's multiple range test. Relationships between variables were quantified by simple regression analyses of plot

percentages. Similar analyses were used at various times to relate mean family performance after artificial inoculation to CPG and NCPG. All tests of statistical significance were at the 0.05 level.

RESULTS AND DISCUSSION

Cumulative disease increase

Plots of CPG against time indicate that few trees were galled during the first 2 years after planting, but that the number of galled trees increased rapidly between the second and sixth seasons, and then more slowly until it approached a maximum within 10 years (fig. 1). Differences between locations are apparent in that the largest increases at Location 2 occurred a year or two after those at Location 1. Nevertheless, maximum CPG was greater at Location 2. Our findings confirm earlier descriptions (Griggs and Schmidt In press) and conform to patterns of disease increase observed in other host/pathogen systems (Kranz 1974).

At both locations, the largest increases occurred when height and crown size were increasing rapidly. Rates of increase lessened thereafter because many new infections occurred on trees already galled. Some susceptible but previously rust-free trees were infected each year, but fewer were as the trial progressed.

Patterns of increase were similar for all families (fig. 1), but degree of infection among families has been significantly different since the 2nd year, and rankings have remained the same or become more definite (table 1). Throughout the trial, Family 1 had significantly fewer galled trees than any other family. Families 5 and 6 were always most frequently galled, while Families 2, 3, and 4 were intermediate. In terms of galls per galled tree, however, Family 2 had nearly three times more potentially sporulating galls 6 years after planting than other intermediate families. The implications are twofold. First, families like Family 2 can be expected to be in greater danger of damage or death than families with similar amounts of infection but fewer galls per tree. Second, such materials may serve to intensify disease by producing more inoculum than others with equivalent infection. This observation also suggests, albeit indirectly, that families with fewer than average galls may occur and that they should be sought for future breeding.

Patterns of increase in CPSG mirror those for CPG with only minor exceptions (fig. 1). The curves are essentially identical until the second or third growing season--an observation not unexpected because planted slash pines have relatively few branches available for infection until then. Also, CPSG levels off later and at lower levels. The slower increases are expected because several years are required for branch galls to grow into the stem.

Family rankings have been stable; differences have been significant and have continued to increase since the 2nd year after planting. At last measurement, Family 1 had the fewest trees with stem galls (table 1), Families 5 and 6 were the most frequently galled, while the others were intermediate.

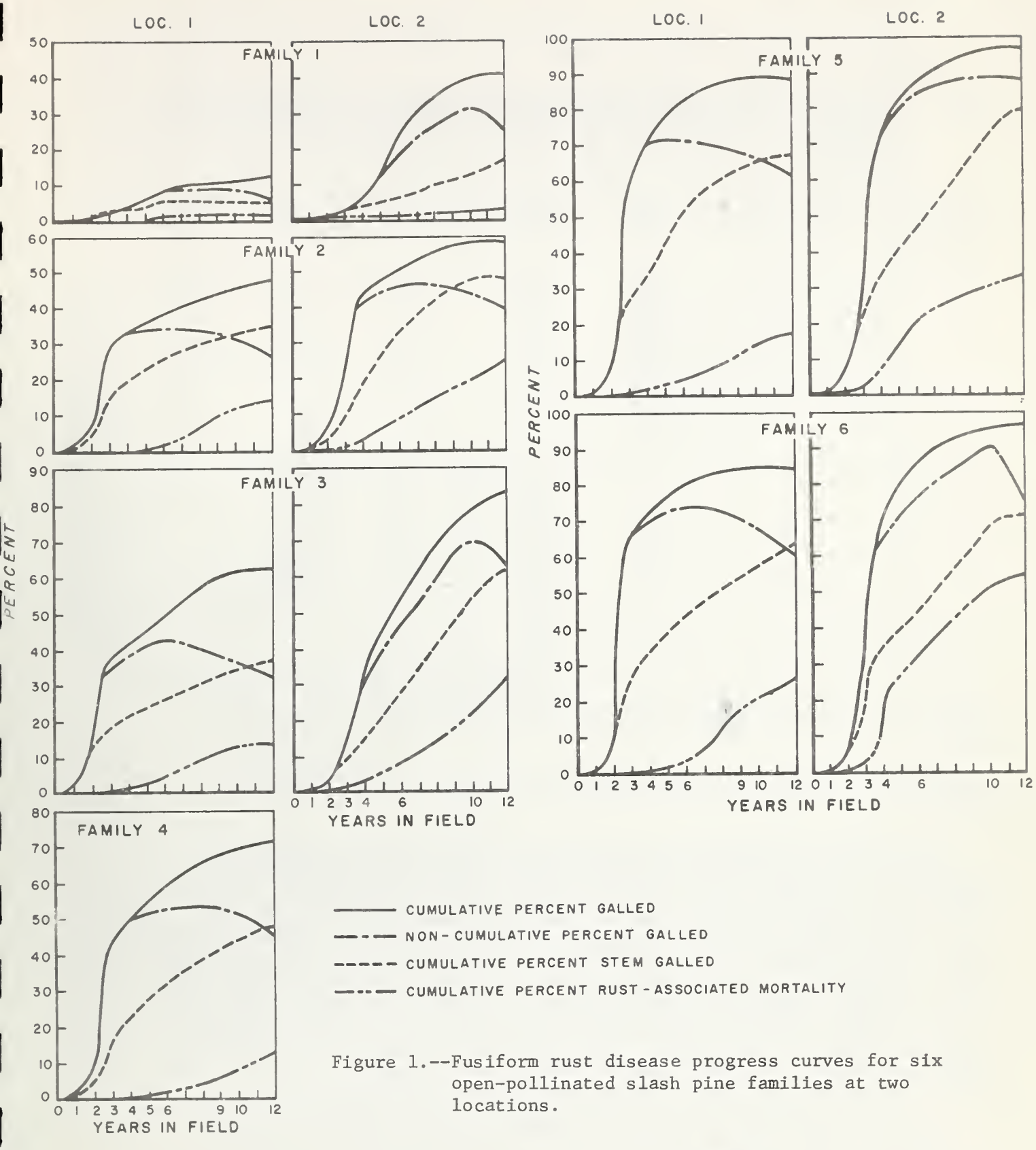

Figure 1.--Fusiform rust disease progress curves for six open-pollinated slash pine families at two locations.

Table 1.--Cumulative and noncumulative estimates (percent) of fusiform rust incidence 12 years after planting and following artificial inoculation

Location	Slash pine family	CPG[a]	CPSG	CPRAM	NCPG	AI[b]
1	1	12	6	1	6	40
	2	46	35	14	27	52
	3	61	35	13	32	95
	4	71	46	12	48	93
	5	88	66	17	62	92
	6	84	60	25	63	94
	$\bar{X}$	61	42	14	38	83
2	1	42	19	3	22	
	2	59	48	26	40	
	3	82	62	32	62	
	5	95	80	34	88	
	6	96	71	55	76	
	$\bar{X}$	78	56	30	52	

[a]Means not connected by the same line differ significantly at the 0.05 level.

[b]Artificial inoculation results (Dinus 1969) apply to both Locations 1 and 2.

The course of CPRAM generally paralleled those of CPG and CPSG, but several differences are apparent (fig. 1). Rust-associated mortality was not observed until several years after the first infections and never reached the same level as CPG and CPSG. Also, the period of most rapid increase began 3 to 4 years after and endured longer than that for infection. The delay and slower increase reflect the time required for stem and branch-associated stem galls to enlarge, girdle, and weaken or kill stems. Moreover, not all stem galls are fatal. Though having plateaued in a few cases, CPRAM probably will increase beyond the 12th growing season, especially in the most heavily infected plantation (Location 2). Other workers have observed continuing rust-associated mortality through 15 or 16 years after planting and expected more deaths thereafter (Jones 1972, Sluder 1977, Wells and Dinus In press).

Families most susceptible in terms of CPG and CPSG generally had the most deaths. In contrast to results for CPG and CPSG, however, family differences were not significant until the fourth growing season at Location 1 and the 10th season at Location 2. Through the 12th year, Family 1 incurred

the least and Family 6 the most deaths (table 1). Family 5 proved intermediate, though it was one of the most susceptible families in terms of CPG and CPSG. In fact, Family 5 had significantly less CPRAM than Family 6 despite having a higher frequency of trees with stem galls. Hence, infected trees within Family 5 withstood and survived infection better than trees in an otherwise equally susceptible family, and as well as families with 20 percentage points less infection in terms of CPG and NCPG (fig. 2). This greater survival resulted in an average plot volume approaching that of families with far less overall infection. Moreover, this tolerance was observed at both locations and was particularly evident in the most heavily infected plantation. These results strongly suggest that tolerance is heritable.

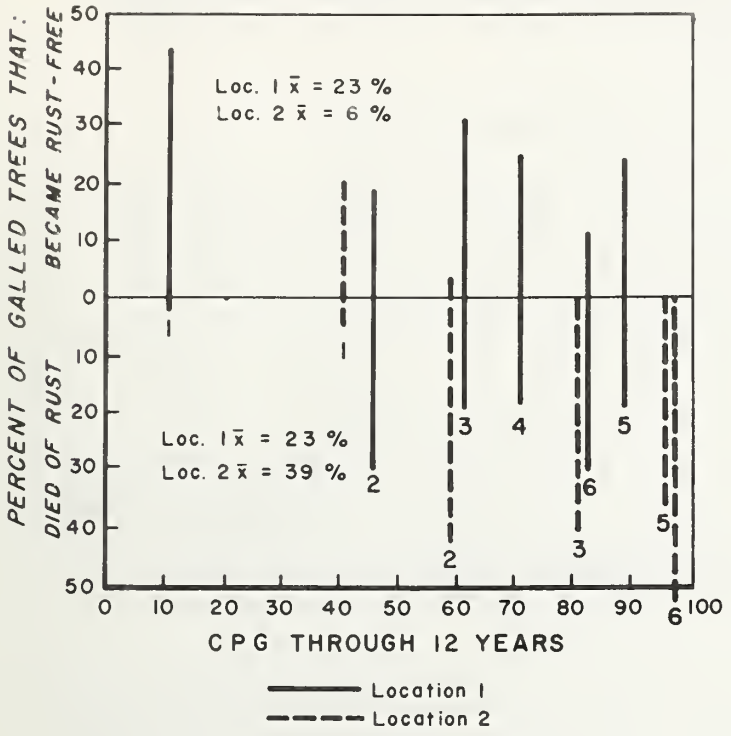

Figure 2.--Effect of rust-associated mortality and natural pruning of galled branches on divergence of noncumulative from cumulative measures (percent) of rust incidence.

The implications are clear--tolerant families should be identified in present and future progeny tests. Once inheritance of tolerance has been confirmed, steps should be taken to incorporate it with other types of resistance now available or becoming available. Such actions seem particularly important because similar phenomena in agronomic crops have proved stable despite shifts in pathogenic virulence (Browning 1974). A word of caution--tolerant trees used alone could intensify disease in certain situations. The greater survival of galled trees could increase inoculum abundance and, consequently, the potential for infection in surrounding plantations.

The overall parallelism of CPG, CPSG, and CPRAM curves implies that early infection may provide a basis for predicting rust-associated mortality.

Simple regressions computed separately for each location support this inference and confirm previous findings (Sluder 1977, and Wells and Dinus In press), though our correlations were not as strong. Variation in CPSG 4 years after planting at Location 1 explained 56 percent of the variation in CPRAM at 12 years. The correlation for Location 2 was 49 percent, also significant. Correlations involving CPG and CPRAM were weaker than for CPSG and CPRAM because CPG includes some trees having only branch galls. Low degrees of freedom precluded analyses within families, but scatter diagrams suggested linear associations for most families. Nature of the relationships, however, appeared to vary among families. For example, the apparent slope for Family 5 was lower than that for Family 6--another indication of the tolerance of Family 5.

Noncumulative disease increase and its relationship to CPG

Plots of NCPG against time indicate that cumulative and noncumulative measures of percent infection generally are identical through the third or fourth growing season (fig. 1). Thereafter, the curves begin to diverge because of rust-associated mortality, natural pruning of galled branches, or some other form of recovery. Both CPG and NCPG continue to increase for several years, but NCPG increases more slowly and eventually declines. This is similar to patterns observed by Schmidt et al. (1974) for slash pine and by Wells and Dinus (In press) for loblolly pine.

Degree of divergence between NCPG and CPG differed between locations and among families (fig. 1 and table 1). Differences in CPRAM account for much of the variation. Infection was heavier at Location 2 and more galled trees died than at Location 1--39 to 23 percent (fig. 2). Also, galled trees in susceptible and intermediate families had a higher probability of death than those in the resistant family.

Divergence also depended upon the frequency with which previously galled trees became and remained rust free, a phenomenon more prevalent at Location 1 than at Location 2 (fig. 2). In four of the six families at Location 1, this form of recovery contributed as much or more to divergence than rust-associated deaths. In contrast, few such trees remained rust free for very long at Location 2 because new infections occurred much more frequently and consistently.

Numbers of galled trees that became and remained rust free also varied among families (fig. 2). This may be because of heritable resistance but, because of difference between sites in environment and inoculum load, it is difficult to assess. Family 1, the most resistant entry, was the only one for which most of the divergency consistently could be attributed to galled trees having become and remained rust free.

Family rankings on the basis of NCPG accurately reflect those for CPG. Few rank changes have occurred in terms of either variable since the second growing season. Hence, relative family resistance can be gauged quickly and inexpensively by point estimates of percent galled.

This consistency also suggests that NCPG would be as accurate a predictor of CPRAM as CPG. Indeed, Wells and Dinus (In press) and Sluder (1977) have found strong correlations between percentage of stem infection observed 5 years after planting and rust-associated mortality at the 10th year in both loblolly and slash pines.

The few rank changes that have occurred were limited to susceptible and intermediate family groups. Such shifts were most prevalent at Location 1 where infection was lighter and more galled trees became and remained rust free. Similar shifts have been observed, particularly in progeny tests involving more families, families with less striking differences in resistance, or low infection rates (Schmidt and Goddard 1971). Our results indicate that CPG may prove more reliable than NCPG in such circumstances and underscore the desirability of establishing progeny tests on hazardous sites (Sohn et al. 1975).

Implications for advanced generation selection

Though relative performance of families can be estimated reliably by cumulative or noncumulative assessments within 3 to 5 years of planting, selecting rust-free individuals within the best families for advanced generation breeding requires much greater care to avoid selecting escapes or previously infected trees that are currently rust free. The potential for each error varies according to amounts of early infection, duration of exposure, and the manner in which rust data are collected.

New infections continue to occur until at least 10 years after planting regardless of family or location (fig. 1). For example, CPG for Family 1 at Location 2 increased from 24 percent in the 6th year to 40 percent in the 10th, showing that 16 of every 100 trees supposedly resistant at year 6 proved susceptible upon further exposure. Early selection based on resistance in field trials therefore does not seem particularly efficient. The danger of selecting escapes was greater at Location 1.

The probability of selecting escapes can be reduced by delaying final selection until 10 or more years after planting. CPG records are then required to avoid the second error of selecting previously galled trees which have become and remained rust free. Divergence of CPG and NCPG curves was not restricted to susceptible families (fig. 1). Some divergence, resulting mainly from loss of galled branches through natural pruning, occurred for even the most resistant Family 1 (fig. 2). These results underscore both the need to maintain histories of disease for individual trees for at least 10 years and the need to establish resistance trials for second generation selection on hazardous sites.

Artificial inoculation and its relationship to CPG and NCPG

Family rankings in terms of both CPG and NCPG also agreed with those from artificial inoculations (table 1). Correlations between family performance at Location 1 and artificial inoculation results have been significant since the 3rd year after planting. The correlation between Location 2 and artificial inoculation results was strong but not significantly so until the 10th year because rapid increase in infection occurred later at

Location 2. Regardless of location, such correlations were strongest for CPG and strengthened with years of exposure.

Variation in results from artificial inoculations explained 81 percent of variation in CPG 12 years after planting at Location 1 as compared with 69 percent of variation in NCPG. At Location 2, artificial inoculation results explained 92 percent of the variation in CPG and 85 percent of that in NCPG. The higher correlations at Location 2 probably result from the tendency of infection levels there to approach those caused by artificial inoculation (table 1).

Our results indicate that artificial inoculations more accurately reflect family rankings than previously supposed (Dinus 1971, Wells and Dinus 1974), especially when field infection is measured and expressed in cumulative terms. That field infection levels eventually match those following artificial inoculations further suggests that in either type of test a similar number of trees would remain rust free and those trees might have similar genotypes. Hence, selection of rust-free survivors from artificial inoculations (Dinus and Griggs 1975) may provide an effective shortcut to field evaluation and selection.

CONCLUSIONS

The foregoing results indicate substantial variation among families in each observed measure of disease incidence. In general, resistant materials can be identified on the basis of percent infection after exposure under artificial or field conditions. Though productive as a first step, such simplified approaches have not taken and cannot take full advantage of the considerable resistance in slash and loblolly pine. An exception is the so-called C-score rating used in some tree improvement programs (Stonecypher et al. 1973). Because numbers of galls per tree and extent of damage are noted, such an index provides more information than the usual procedures. Even these ratings, however, are averages and do not allow breeders to find, isolate, and intercross materials with different forms of resistance.

Detailed evaluation of disease progress curves can identify parents with different types of resistance, including tolerance, and clarify the significance of their resistance in the epidemiology of fusiform rust. Once the nature and inheritance of different resistance types are understood, planned combinations can be made to insure that future seed orchards and plantations contain a variety of resistance genes.

LITERATURE CITED

Browning, J. A. 1974. Relevance of knowledge about natural ecosystems to development of pest management programs for agro-ecosystems. Proc. Am. Phytopathol. Soc. 1: 191-199.

Dinus, R. J. 1969. Testing slash pine for rust resistance in artificial and natural conditions. In Proc. 10th South. For. Tree Improv. Conf., p. 98-106.

Dinus, R. J. 1971. Phenotypic selection for fusiform rust resistance. In Proc. 11th South. For. Tree Improv. Conf., p. 68-75.

Dinus, R. J., and M. M. Griggs. 1975. Rust-free slash pines surviving artificial inoculation potentially useful for resistance breeding. For. Sci. 21: 275-277.

Dinus, R. J., G. A. Snow, A. G. Kais, and C. H. Walkinshaw. 1975. Variability of Cronartium fusiforme affects resistance breeding strategies. In Proc. 13th South. For. Tree Improv. Conf., p. 193-196.

Griggs, M. M., and R. A. Schmidt. (In press). Increase and spread of fusiform rust. In Fusiform Rust of South. Pine: Dis. Manage. Strategies Symp., Gainesville, Fla.

Jones, E. P., Jr. 1972. Fusiform rust affects planted slash pine. J. For. 70: 350-352.

Kranz, J. 1974. Epidemics of plant diseases: mathematical analysis and modeling. Springer-Verlag, New York. 170 p.

Rockwood, D. L., and R. E. Goddard. 1973. Predicted gains for fusiform rust resistance in slash pine. In Proc. 12th South. For. Tree Improv. Conf., p. 31-37.

Schmidt, R. A., and R. E. Goddard. 1971. Preliminary results of fusiform rust resistance from field progeny tests of selected slash pines. In Proc. 11th South. For. Tree Improv. Conf., p. 37-44.

Schmidt, R. A., R. E. Goddard, and C. A. Hollis. 1974. Incidence and distribution of fusiform rust in slash pine plantations in Florida and Georgia. Fla. Agric. Exp. Stn. Bull. No. 763.

Sohn, E. I., R. E. Goddard, and R. A. Schmidt. 1975. Comparative performances of slash pine for fusiform rust resistance in high rust hazard locations. In Proc. 13th South. For. Tree Improv. Conf., p. 204-211.

Sluder, E. R. 1977. Fusiform rust in loblolly and slash pine plantations on high-hazard sites in Georgia. USDA For. Serv. Res. Pap. SE-160, 10 p. Southeast. For. Exp. Stn., Asheville, N.C.

Stonecypher, R. W., B. J. Zobel, and R. Blair. 1973. Inheritance patterns of loblolly pines from a nonselected natural population. N.C. Agric. Exp. Stn. Tech. Bull. No. 220, 60 p.

Wells, O. O., and R. J. Dinus. 1974. Correlation between artificial and natural inoculation of loblolly pine with southern fusiform rust. Phytopathology 64: 760-761.

Wells, O. O., and R. J. Dinus. (In press). Early infection as a predictor of mortality associated with rust of southern pines. J. For. 75.

Wells, O. O., and P. C. Wakeley. 1966. Geographic variation in survival, growth, and fusiform-rust infection of planted loblolly pine. For. Sci. Monogr. 11, 40 p.

WIDE CROSSES IN THE SOUTHERN PINES

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and Bruce J. Zobel 1/

Abstract.--Previous efforts to produce hybrids between members of the genus Pinus have resulted in a "set of rules" as to what can and cannot be crossed. Disregarding these rules, all possible crosses were attempted, especially those with the southern pines. The available seed were planted, and morphological characters prevalent from time of planting through one year's growth were measured.

The objective of this paper is to report on early performance of the putative hybrids compared to parental species. No claim of hybridity is made; however, intermediacy of the crosses compared to the parent species is suggested to be an indication of hybridity in some implausible crosses. Further assessment of hybridity will take place in the field plantings. Performance and putative hybridity is commented on by cross, followed by a brief summary.

Additional keywords: Interspecific, putative hybrids

The possibility of making hybrids amongst members of the genus Pinus has intrigued researchers for many years and has been explored more fully than in most genera of forest trees (Critchfield, 1975). The effort expended in this activity has resulted in a "set of rules" as to what can be crossed and what cannot. How these rules are applied depends on which of the several authorities on nomenclature one follows. The more common of these are listed in Table 1 (page 17) of Dorman (1976); others are listed in Duffield (1952) and Little and Critchfield (1969). An excellent listing of the species involved by group or section is given in Mirov (1967), starting on page 521. We are following Duffield's (1951) classification which divides the subgenus Diploxylon into groups. 2/

The general rule is that crossing among groups generally cannot be done; this is expressed by Critchfield (1975), "crossing is usually impossible among the 15 groups of species (subsections) currently recognized as making up the genus Pinus." There are some exceptions in which intergroup crosses were successful, as listed in Table 1 of Critchfield (1975); only two of these represent southern pines. These crosses are P. elliottii x P. clausa (Saylor and Koenig, 1967) and P. taeda x P. clausa (Critchfield, 1963). As a result of the rule

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2/ These are equivalent to subsections.

and the few successful crosses between groups, many crosses have not been tried and even scoffed at as "ridiculous to try" by some biologists.

In 1959 Hoerner-Waldorf started a pine arboretum in the Coastal Plain of North Carolina. Through the years, 80 species and 10 varieties were planted; of these, 50 survived. Many of these grew well enough to produce abundant flowers, some at an early age.

At that time the question arose as to the feasibility of making selective crosses among the flowering species. The decision was made to ignore the "rules," to throw away the book and try all possible crosses, especially those with any of the southern pines. Such crossing has been pursued over a number of years; in 1974 and 1975 the seed available were planted in the greenhouse, then moved into field plantings at Hoerner-Waldorf's Research Center at Tillery, North Carolina.

The objective of this paper is to report on early performance of the putative hybrids in the greenhouse, compared to parental species. No claim is made that the crosses are truly hybrids; final determination will be made after the trees planted in the field are older, based upon morphological, anatomical, flowering and isozyme analyses. The present paper will point out intermediacy of the crosses grown in the greenhouse as an indication of hybridity in some implausable crosses not expected to be successful based upon the rules.

MATERIALS AND METHODS

This study of parents and putative across-group pine hybrids was a joint effort of the Pine Cooperative at North Carolina State University and Hoerner-Waldorf Corporation of Roanoke Rapids, North Carolina. The crosses in the arboretum were made by Ray Brown at Tillery, North Carolina. The origin for each parent species is shown in Table 1. Species verification is not absolute and the nomenclature supplied by the seed supplier was accepted unless there was an obvious error or suspicion of mislabeling. As far as we know, each species was represented by a number of parents. Sixteen trees of each species were planted in a block in the arboretum. Most crosses made involved several female parents and a pollen mix from several parents, except when flowering was limited to one or two trees within the species block. A few of the seed lots were extracted at Raleigh, North Carolina, but most were extracted at Tillery and sent to North Carolina State University for planting in the greenhouse. When large enough, the trees were taken to Tillery for establishment in the hybrid plantation.

The seed from each lot were subjected to a 30-day stratification. They were then treated with arasan and planted in small peat pots in the greenhouse at the Genetics Nursery at North Carolina State University. A soil mixture of two parts humus soil, one part sand, and one part vermiculite was used as the planting medium. A light layer of fine sand was spread over the pots and a periodic mist begun at time of seeding. The temperature in the greenhouse was kept in the 70° - 80° (F.) range.

A small number of seedlings was lost to damping off at an early age. Several applications of 50 percent Captan in water solution were made to control the disease which was prevalent because of excess moisture. There was

Table 1.--Origin of the parents used to produce the putative hybrids

Species	Common name	Group[a/]	Origin
P. echinata	shortleaf pine	Australes	Widespread in southeastern U. S.
P. pungens	table-mountain pine	Australes	Mountains of southeastern U. S.
P. rigida	pitch pine	Australes	Eastern U. S. highlands in its southern range
P. serotina	pond pine	Australes	Southeast U. S., coastal sites
P. taeda	loblolly pine	Australes	Widespread in southeastern U. S.
P. banksiana	jack pine	Contortae	Northeast U. S. & Canada
P. clausa	sand pine	Contortae	Florida and southern Alabama
P. virginiana	Virginia pine	Contortae	Southeastern U. S., primarily in uplands in its southern range
P. pinaster	maritime pine	Sylvestres	Southwest Europe and northwest Africa
P. thunbergii[b/]	Japanese black pine	Sylvestres	Japan and Korea
P. densiflora	Japanese red pine	Sylvestres	Japan

[a/] Group and subsection are used interchangeably.

[b/] This species has recently been renamed P. thunbergiana.

some wilting such as noted by Franklin (1969) in which the cotyledons wilted and then the seedling died two to three weeks after germination. This may have been caused by the conditions in the greenhouse or possibly by some physiological breakdown which prevented water uptake and further development. 3/

One trend noted in putative hybrids of both plantings was the tendency for the seed coats to adhere to the cotyledons. When not shed naturally, the seedlings often died. When the seed coats were removed manually, the cotyledons were twisted and misshapen but usually recovered and appeared to start normal growth within a few days.

The morphological characteristics examined were those that are evident from the time the seeds are planted until the end of the first growing season. Germination speed and characteristics were recorded daily, as was secondary needle formation later in the year.

3/ Personal communication, Dr. R. C. Kellison, N. C. State University.

The number of cotyledons and length of the hypocotyl were recorded periodically to determine number, rate of development and other characteristics. Height measurements were taken for the mean for each parent or putative cross after one growing season (approximately six months). Height was accurately measured from the soil surface to the tip of the apical bud and appears to be a most useful indicator of hybridity. Hypocotyl color differences were great and always observed on the same number of days after germination for parents and putative crosses; this was a subjective measurement, however, and its usefulness as an indicator of hybridity could sometimes be questioned.

Many crosses were attempted with each parent species in the arboretum. The 29 putative hybrids with sufficient seed to plant are listed in Table 2 with the groups they represent. As listed, there are seven intragroup crosses and 22 intergroup crosses; only P. taeda x P. clausa (Critchfield, 1963) has been reported as being successful. The only other successful intergroup cross reported is P. elliottii x P. clausa (Saylor and Koenig, 1967).

RESULTS AND CONCLUSIONS

No claim of hybridity can be made solely on greenhouse tests. In most cases, however, the putative cross exhibits morphological characteristics of the seedlings that are distinctly different from seedlings of either parent species. These differences, which are often intermediate, can be seen from Table 2 by comparing each putative cross with its respective parents. Outstanding examples of differences or intermediacy are commented upon in the following paragraphs with comments about some unusual features observed. Space does not enable discussion of every cross.

The strongest characteristics denoting hybridity in the intergroup cross P. thunbergii x P. pungens was the reduced average height of the cross after one growing season. The lesser height may indicate physiological incompatibility between P. pungens (group Australes) and P. thunbergii (group Sylvestres). The rate of secondary needle formation was similar to the female parent P. thunbergii.

The intergroup cross P. taeda x P. pinaster showed some characteristics different from the parents. The average height of the cross was less than either parent, and the time required for germination and secondary needle formation was much longer than for both parent species. The reciprocal cross P. pinaster x P. taeda showed similar tendencies as P. taeda x P. pinaster but seedlings exhibited a strong reduction in chlorophyll content, having nearly white needles and light pink hypocotyls. This was retained after outplanting.

P. clausa x P. virginiana was intermediate in most characteristics measured. Most outstanding was the intermediate height obtained from a considerable number of seedlings of both parents and the cross. No abnormal seedlings were noted. The reciprocal cross P. virginiana x P. clausa was intermediate in some measurements but heights were similar and hypocotyl color was intermediate. Based on greenhouse observations, both of these within-group crosses appear to be good candidates for true hybrids.

Table 2.--Comparison of physical characteristics of parents and crosses[2/]

Cross and parents	Number seed planted	Number seed germ.	Number cotyledons	Color of hypocotyl[c/]	Length of hypocotyl (cm)	Avg. Ht. (1 growing season)(cm)	Secondary needle formation (days)	Groups crossed d/
				1974 Planting				
P. clausa	50	34	5 - 8	lt. red	1.6 - 3.2	14.4	69	
P. clausa x P. virginiana	50	24	5 - 7	lt. red	1.2 - 4.2	8.2	92	C x C
P. virginiana	50	37	5 - 7	lt. red	2.0 - 3.0	6.1	97	
P. pinaster	50	30	6 - 9	lt. purple	2.0 - 2.8	15.2	62	
P. pinaster x P. echinata	50	35	7 - 10	lt. red-purple	1.3 - 3.0	12.9	75	S x A
P. echinata	50	35	5 - 7	red	0.7 - 2.0	7.9	83	
P. pinaster x P. pungens	50	37	7 - 10	deep purple	2.0 - 3.3	17.2	64	S x A
P. pungens	50	13	7 - 8	lt. red	2.0 - 2.8	6.8	83	
P. pinaster x P. serotina	50	21	6 - 9	deep purple	2.0 - 3.3	13.3	82	S x A
P. serotina	50	42	5 - 8	pink	0.5 - 2.1	14.4	76	
P. taeda	50	33	6 - 8	red	2.5 - 4.3	15.8	70	
P. taeda x P. pinaster	50	6	7	red	3.0 - 4.8	11.8	85	A x S
P. taeda x P. thunbergii	50	1	8	red	3.8	9.0	68	A x S
P. thunbergii	50	27	6 - 8	pale red	1.5 - 2.5	5.7	65	
P. taeda x P. virginiana	50	19	6 - 8	red	2.5 - 4.1	12.2	89	A x C

Cross and parents	Number seed planted	Number seed germ.	Number cotyledons	Color of hypocotyl [c/]	Length of hypocotyl (cm)	Avg. Ht. (1 growing season) (cm)	Secondary needle formation (days)	Groups crossed [d/]
				1974 Planting				
P. thunbergii x P. clausa	50	22	5 - 8	lt. green	1.7 - 3.0	8.8	60	S x C
P. thunbergii x P. pungens	50	30	7 - 9	lt. green	2.0 - 3.0	4.9	65	S x A
P. thunbergii x P. serotina	50	27	5 - 8	pink	1.3 - 2.8	7.2	68	S x A
P. thunbergii x P. taeda	50	12	6 - 8	green	1.5 - 2.7	5.0	76	S x A
P. taeda x P. clausa	50	0						A x C
				1975 Planting				
P. pinaster x P. taeda	27	4	7	red	a/	10.8	128+ [b/]	S x A
P. rigida	75	58	5 - 7	lt. red	2.5 - 3.2			
P. rigida x P. clausa	17	8	5 - 7	lt. red	1.1 - 2.5	8.8	99	A x C
P. taeda x P. banksiana	36	4	4 - 6	a/	0.2 - 1.0	3.2	128+ [b/]	A x C
P. taeda x P. clausa	49	3	6 - 8	red	1.7 - 3.8	7.8	128+ [b/]	A x C
P. taeda x P. serotina	100	85	4 - 7	pale red	0.5 - 2.3	10.4	128	A x A
P. taeda x P. virginiana	100	78	5 - 8	purple	1.8 - 5.0	14.3	109	A x C
P. thunbergii x P. rigida	33	5	6 - 8	pink	2.3 - 3.0	a/	91	S x A

Cross and parents	Number seed planted	Number seed germ.	Number cotyledons	Color of hypocotyl[c/]	Length of hypocotyl (cm)	Avg. Ht. (1 growing season)(cm)	Secondary needle formation	Groups crossed [d/]
				1975 Planting				
P. virginiana x P. banksiana	100	58	5 - 7	red-orange	2.0 - 3.5	5.2	128+[b/]	C x C
P. virginiana x P. clausa	100	73	4 - 7	pale red	1.0 - 2.7	5.7	109	C x C
P. virginiana x P. echinata	36	4	5 - 7	pale red	1.5 - 3.0	4.5	128+[b/]	C x A
P. virginiana x P. pinaster	32	5	5 - 8	red	2.0 - 3.0	7.4	128	C x S

The following crosses did not produce any seedlings:

	Number of seed	Groups crossed
P. echinata x P. banksiana	31	A x C
P. echinata x P. pinaster	45	A x S
P. densiflora x P. rigida	43	S x A
P. densiflora x P. taeda	30	S x A

[a/] Value not obtained

[b/] Approximate value

[c/] This was a subjective measurement.

[d/] Group designations: A = Australes, C = Contortae, S = Sylvestres.

The unexpected intergroup cross P. thunbergii x P. taeda shows many of the characteristics of the parent P. thunbergii with the exception of the time required for secondary needle formation, which was a little longer than for either parent. Because of the resemblance to the female parent, the hybridity of this cross is rather doubtful. However, the reciprocal cross P. taeda x P. thunbergii, of which only one of 50 seeds germinated, showed intermediacy in most measurements. One seedling is insufficient to make any statement of hybridity even if it has intermediate characteristics.

The intergroup cross P. thunbergii x P. serotina was intermediate in average height and somewhat so for time required for secondary needle formation. One dwarf seedling was noted that had a thickened hypocotyl and never developed primary needles; it was outplanted when approximately five centimeters in height. Characteristics of seedlings and fairly good seed germination show definite indications of hybridity.

P. pinaster x P. pungens, an intergroup cross, appeared to have characteristics different from the parents; it had much the same form as P. pinaster. The average height of the cross is somewhat misleading because some of the seedlings of the cross were as much as fifteen centimeters taller than the parent P. pinaster. These tall seedlings produced primary needles along approximately three-fourths of the stem, had good straight form, and were vastly superior to the P. pungens seedlings. This could well be a hybrid. Percent germination was much better than both parents.

The intergroup cross P. thunbergii x P. clausa was intermediate in height between the parents and the color of the hypocotyl was very different from the parents; secondary needle formation was faster than for either parent. We feel this is a good cross.

The intergroup cross P. taeda x P. virginiana was established in both years' plantings. It exhibited some intermediate and some characteristics of each parent in both tests. Hybridity at this stage is uncertain but there are enough characteristics like the pollen parent that hybridity may be present. There were four abnormal seedlings which had short, hard, thickened hypocotyls and did not appear to produce any new growth after the primary needles were formed. If confirmed, this putative hybrid will be of special interest since the P. taeda x P. virginiana cross has been tried by several persons without apparent success.

Except for cotyledon number the intergroup cross P. pinaster x P. echinata has intermediate characteristics in color of the hypocotyl, average height and time required for secondary needle formation. This appears to be most positive of the many putative hybrids grown; even percent seed germination was good. No seed germinated from the reciprocal P. echinata x P. pinaster. The significance of this is mentioned later.

P. pinaster x P. serotina, another intergroup cross, appears to be a hybrid; at least its characteristics were sometimes much different from P. pinaster. The average height was less than for either parent, and the time required for secondary needle formation was considerably longer. No abnormal seedlings were noted but percent of germination was quite good.

The cross P. virginiana x P. banksiana showed a marked contrast from either parent. Intermediacy was evident in every measurement except secondary needle formation. The time for secondary needle formation was much longer than for either parent. This is a good candidate for a hybrid as an intragroup cross.

P. taeda x P. serotina looks very much like the male parent. All measurements are similar to the P. serotina, indicating hybridity. This hybrid would be expected but the direction toward the male, rather than intermediacy, was a surprise.

The intergroup cross P. virginiana x P. echinata has some characteristics of P. echinata but has depressed height growth. The lack of clear-cut differences or intermediacy makes it difficult to assess the hybridity of this cross.

The cross P. taeda x P. banksiana responded as crosses between groups often do. Only four seed germinated from the 100 that were planted; secondary needle formation was very slow on the survivors, and the length of the hypocotyl and the average height were less than for either parent. The small number of seedlings does not allow a statement of hybridity but it appears from the characteristics of the four seedlings that this is a hybrid.

An historically unsuccessful intergroup pine cross, P. taeda x P. clausa, has produced only seedlings that died soon after germination (Critchfield, 1963). The first planting of this cross also produced no seedlings, but the second planting produced three seedlings from 49 seed, including one albino. There appears to be considerable intermediacy and deviation from either parent. These three seedlings were field planted; the albino seedling acquired some green pigmentation in the field planting.

P. virginiana x P. pinaster may be a successful intergroup cross. The strongest characteristic denoting hybridity was its intermediate height; also it varied greatly in time required for secondary needle formation. Some of the traits of P. pinaster are seen in the putative cross. The number of cotyledons and much longer time required to produce secondary needles reinforce the suspicion of hybridity.

The intergroup crosses P. rigida x P. clausa and P. thunbergii x P. rigida appear to be successful hybrids; however, there was insufficient information obtained to come to a conclusion. The color of the hypocotyl was intermediate in both crosses, but values for other characteristics are not very informative.

There were five intergroup crosses for which seed did not germinate. P. echinata x P. banksiana, P. echinata x P. pinaster, P. densiflora x P. taeda, and P. densiflora x P. rigida yielded no seedlings. P. taeda x P. clausa yielded no seedlings in the first planting but three seeds germinated in the second planting. This does not prove success or failure but nongermination has been unusual in many crosses between different groups.

In his new book, Dorman (1976) has a section outlining hybridity among taxonomic groups of pines, with emphasis on the southern pines. A cross of P. silvestris x P. palustris has been reported but is considered to be doubtful. Similarly, Dorman states that a P. taeda x P. densiflora cross in 1950

is suspect. Crosses of P. contorta x P. pungens and P. densiflora x P. rigida have been made but hybridity is as yet unproved. The hybrid of P. rigida x P. radiata has been made and reported numerous times in Korea.

Of the intragroup crosses made, most appeared to be hybrids; of the 20 intergroup crosses, 12 appear to be good candidates, seven are doubtful but differ from the parents, and one appears to be a noncross. It will be very interesting to confirm or reject these findings after the trees have been grown about five years in the field and a multidiscipline attack to hybridity has been made.

SUMMARY

Many seemingly impossible crosses were made on pines in the Hoerner-Waldorf arboretum at Tillery, North Carolina. Some of these crosses produced seed. These seed were planted in the greenhouse where many measurements and observations were made. No claim of hybridity is made, based on these greenhouse tests; however, several intergroup crosses are offered as putative hybrids based on physical measurements and observations.

LITERATURE CITED

Critchfield, W. B. 1963. Hybridization of the southern pines in California. Southern Forest Tree Improvement Committee, Publication 22. pp. 40-48.

Critchfield, W. B. 1975. Interspecific hybridization in Pinus: A summary review. Symposium on Interspecific and Interprovenance Hybridization in Forest Trees. Proc., 14th Meet., Canada Tree Impr. Assn. Part 2:99-105.

Dorman, K. W. 1976. The genetics and breeding of southern pines. Agri. Handbook No. 471, USDA, Forest Service. 407 pp.

Duffield, J. W. 1952. Relationships and species hybridization in the genus Pinus. Z. Forstgen. 1:93-100.

Franklin, E. C. 1969. Mutant forms found by self-pollination in loblolly pine. Jour. of Heredity 60(6):315-320.

Little, Jr., E. L. and W. B. Critchfield. 1969. Subdivisions of the genus Pinus (Pines). USDA, Forest Service Misc. Pub. No. 1144. 51 pp.

Mirov, N. T. 1967. The genus Pinus. Ronald Press, New York. 602 pp.

Saylor, L. C. and R. L. Koenig. 1967. The slash x sand pine hybrid. Sil. Gen. 16:134-138.

THIRD-YEAR RESULTS OF A SHORTLEAF X LOBLOLLY PINE HYBRID PROGENY TEST IN GEORGIA

Timothy La Farge and John F. Kraus[1/]

Abstract.--A 3-year progeny test of shortleaf X loblolly pine hybrids bred to recombine the high resistance of shortleaf pine to fusiform rust with the rapid growth rate of loblolly pine confirms the findings of an earlier artificial inoculation study. Progeny of selected F2 hybrids backcrossed to loblolly pine were significantly more resistant than loblolly but equalled, and in some backcrosses exceeded, it in growth rate. Similarly, F1 hybrids and progeny of wind-pollinated F2 hybrids were significantly faster growing than shortleaf pine but retained the same high level of resistance to rust.

Additional keywords: Backcross, Pinus echinata, P. taeda, Cronartium fusiforme, recombine.

In 1975 we reported promising results from an artificial inoculation test of hybrid crosses between loblolly pine (Pinus taeda L.) and shortleaf pine (P. echinata Mill.). That study was designed to determine growth and resistance of the hybrids to fusiform rust (Cronartium fusiforme Hedgc. and Hunt ex Cumm.) (La Farge and Kraus 1975). Those results indicated that rust resistance and growth rate might be recombined. However, since the seedlings were only 9 months old when measured, inferences concerning growth rate were inconclusive. This paper reports supporting evidence from a progeny test after 3 years in the field.

The present study is larger than the former; it consists of 30 seedlots representing 3 different hybrid types and both parent species. The former study comprised only 12 seedlots representing 3 hybrid groups and one parent species (loblolly). The original 12 seedlots are included in the present study. Since this is a field test, it also offers an opportunity to compare natural infection with artificial inoculation on those seedlots which were common to both studies.

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MATERIAL AND METHODS

The material consists of the following five groups: (1) Group 1, open-pollinated shortleaf; (2) Group 2, shortleaf X loblolly F1 hybrids; (3) Group 3, the progeny of wind-pollinated shortleaf X loblolly F2 hybrid selections; (4) Group 4, the progeny of selected shortleaf X loblolly F2 hybrids back-crossed to loblolly; and (5) Group 5, open-pollinated loblolly. Crosses included in each group are shown in table 1.

Table 1.--Summary of traits measured at age 3 years in a progeny test of shortleaf X loblolly pine hybrids in Houston County, Georgia

Groups and seedlots in groups	Height	Trees free of rust
	Feet	Percent
Group 1 (Progeny of Wind-pollinated shortleaf)		
TVA X Wind	4.6	100
Z15 X Wind	4.2	100
Piedmont Commercial	3.9	100
Group mean	4.3	100
Group 2 (F1 Hybrids)		
Z15 X 541	5.2	100
Z15 X 536	5.0	100
Z15 X 631	5.3	94
Group mean	5.1	98
Group 3 (Progeny of Wind-pollinated F2 Hybrids)		
HH 8 X Wind	5.6	100
HH 20 X Wind	4.9	100
HH 5 X Wind	4.9	100
HH 6 X Wind	4.7	98
HH 15 X Wind	5.2	92
Group mean	5.1	98

Table 1.--(Cont'd)

Groups and seedlots in groups	Height	Trees free of rust
	Feet	Percent
Group 4 (Progeny of F2 Hybrids Backcrossed to Loblolly)		
HH 19 X 624	5.6	97
HH 19 X 607	6.0	94
HH 5 X 624	6.5	97
HH 5 X 520	5.7	88
HH 11 X 607	4.8	95
HH 11 X 515	5.6	85
HH 17 X 518	6.0	92
HH 17 X 541	5.5	83
HH 15 X 541	5.9	94
HH 15 X 600	5.5	79
HH 8 X 520	6.5	86
HH 8 X 600	6.4	85
HH 30 X 603	6.2	71
HH 13 X 603	5.8	71
HH 13 X 518	5.6	71
HH 6 X 617	6.0	78
HH 6 X 515	6.0	45
Group mean	5.8	83
Group 5 (Progeny of Wind-pollinated Loblolly)		
GCIA 2G-9-5-3	5.9	47
GCIA 2G-65-D1	6.0	38
Group mean	6.0	42

The wind-pollinated parents of the trees in Group 1 had three origins:

1. Z15, a superior tree in Harris County, Georgia. This tree's progeny have demonstrated superior resistance to littleleaf disease, caused by Phytophthora cinnamomi Rands, in tank tests (Zak 1955) and in the laboratory (Bryan 1965). Its offspring have shown some resistance to adverse soil conditions plus P. cinnamomi as well as P. cinnamomi alone, and Z15 progeny had exceptional height growth in field tests (Bryan 1973).
2. Three clones in the Georgia Forestry Commission (GFC) seed orchards. These clones originated from ortets selected by the Tennessee Valley Authority.
3. Shortleaf pine seed commercially collected from the Georgia Piedmont.

Group 2 consists of three single crosses between Z15 and three GFC seed orchard clones of loblolly pine as pollen parents.

Group 3 comprises the progeny of five wind-pollinated mother trees which were selected F2 hybrids. The F1 parents of these hybrids were the offspring of shortleaf from North Carolina and loblolly from Virginia and were grown by the Institute of Forest Genetics in Placerville, California.

Group 4 is composed of 17 single crosses between selected F2 hybrids (those comprising Group 3 and others from the same source) and selected loblolly GFC seed orchard clones as pollen parents.

Two Georgia Crop Improvement Association (GCIA) commercial check lots of loblolly pine make up Group 5 (table 1).

In November 1973 the seeds were germinated in the laboratory, and within 2 days after germination each seedling was transplanted to a peat pot in the greenhouse. The peat pots were placed in cedar flats so as to form 4 x 5 = 20-seedling rectangular plots. The study occupied five benches in the greenhouses, each bench representing one replication.

The seedlings were planted in late June 1974 in Houston County, Georgia, south of Route 26. The site is typical of the Upper Coastal Plain. The use of peat pots made such a late planting possible. After three growing seasons survival was 94.7 percent.

The plantation was measured in late January 1977. Total height and the numbers of stem and branch galls were recorded for each tree.

The variables analyzed were height and arcsin $\sqrt{\text{percent}}$ of trees free of rust. The test was arranged in a randomized complete-block design with 5 replications. There were 30 seedlots and 16 trees in each square plot. Differences tested among groups and among seedlots within groups were planned orthogonal comparisons. These differences were tested for statistical significance at the 0.01 level, and the results of these tests are summarized in table 2.

RESULTS AND DISCUSSION

As in the artificial inoculation test, the loblolly controls in Group 5 had by far the lowest percentages of trees free of rust (table 1). In the 3-year field test the larger sample of 17 progenies of F2 hybrids backcrossed to loblolly (Group 4) still maintained essentially the same average growth rate as the loblolly controls. In fact, table 2 shows that Groups 4 and 5 did not differ significantly for height growth but were statistically different at the 1% level for the percentage of trees free of rust.

Table 2.--Analysis of variance of orthogonal comparisons among progenies and groups of hybrids in a progeny test of shortleaf X loblolly pine hybrids in Houston County, Georgia

Source of variation	Degrees of freedom	Mean squares	
		Height	Trees free of rust
Block	4	7.48**	117.64
Progeny	29	2.09**	839.41**
Within Group 1	2	0.64	0.30
Within Group 2	2	0.11	47.28
Within Group 3	4	0.54	68.76
Within Group 4	16	0.87**	483.26**
Within Group 5	1	0.04	61.31
Group 1 vs. Groups 2 + 3	1	7.54**	35.20
Group 2 vs. Group 3	1	0.06	1.20
Groups 1 + 2 + 3 vs. Groups 4 + 5	1	35.15**	9,409.92**
Group 4 vs. Group 5	1	0.16	6,732.91**
Block X Progeny (Error)	112	0.25	60.78

** Difference is statistically significant at the 0.01 level.

The differences among families within Group 4 were also highly significant for both traits (table 2). Four of the families in Group 4 exceeded the loblolly controls in height, and three of these exceeded the group average in the percentage of trees free of rust. There is only one family, HH 6 X 515, which performed as poorly for this trait as the loblolly controls. The other 16 families exceeded the controls by at least 24 percent. Conversely, only two of the six families in Group 4 that had at least 90 percent of trees free of rust were surpassed by the loblolly controls in height growth. This was the only group within which differences were significant.

Gains in resistance to rust and growth rate were not limited to the backcrosses in Group 4. The F1 hybrids (Group 2) and the progeny of wind-pollinated F2 hybrids in Group 3 were significantly taller than the pure shortleaf in Group 1 but did not differ from that group in resistance to fusiform rust. However, the F1 hybrids of Group 2 did not differ significantly from the progeny of the wind-pollinated F2 hybrids of Group 3 for height growth or rust resistance.

One other planned orthogonal comparison was statistically significant for each trait. The progeny of wind-pollinated shortleaf, the F1 hybrids, and the progeny of wind-pollinated F2 hybrids (Groups 1, 2 and 3) collectively were slower growing and more rust resistant than the progeny of F2 hybrids backcrossed to loblolly and the loblolly controls (Groups 4 and 5). The only meaningful result of this comparison is that it places Group 4 in association with the loblolly control. Yet, as we have already seen, most of the crosses in Group 4 had considerably less rust than the loblolly controls. Hence, the progeny of F2 hybrids backcrossed to loblolly (Group 4) seem to represent the desired products of the hybrid breeding strategy. They are the beginnings of a new strain of loblolly pine with that species' desirable growth but also with resistance to fusiform rust.

These results were generally similar to those of the smaller inoculation study (La Farge and Kraus 1975). To determine more precisely the degree of similarity, we ran simple correlations between the percentages of trees that were galled in the inoculation test and the percentages of trees free of rust in the field test. Only the 12 crosses common to both tests were included. Note that a favorable correlation will be negative because of the difference in the way the same trait was measured in each test. For all 12 families, representing Groups 2, 3, 4 and 5, the test-to-test correlation was $r = -0.83$ (significant at the 1% level). When we based the correlation on only those 8 families in Group 4, the progeny of F2 hybrids backcrossed to loblolly, the correlation coefficient was $r = -0.80$ (significant at the 5% level). These correlations agree with results reported by Dinus (1972), who obtained a very close similarity in relative responses to artificial inoculation and field infection of six half-sib slash pine (P. elliottii Engelm.) families.

CONCLUSIONS

The results of this study support the conclusion of the earlier inoculation test: resistance to fusiform rust may be transferred from shortleaf to loblolly pine without reducing growth rate. Since the present study is older and contains more groups and seedlots within groups, this conclusion is more firmly established by the existing data. However, such a conclusion cannot be considered fully reliable until these trees are at least 10 years old. By then we will have additional hybrid material in the field to supplement our backcrossing program.

LITERATURE CITED

Bryan, W. C. 1965. Testing shortleaf pine seedlings for resistance to infection by Phytophthora cinnamomi. USDA Forest Serv. Res. Pap. SE-50, 4 p. Southeast. For. Exp. Stn., Asheville, NC.

Bryan, W. C. 1973. Height growth of shortleaf pine progenies from trees selected for resistance to littleleaf disease. USDA For. Serv. Res. Note SE-185, 7 p. Southeast. For. Exp. Stn., Asheville, NC.

Dinus, Ronald J. 1972. Testing for fusiform rust resistance in slash pine. In Biology of rust resistance in forest trees, p. 331-339. NATO-IUFRO Adv. Study Inst. Proc., USDA Misc. Publ. 1221.

La Farge, Timothy, and John F. Kraus. 1975. Resistance of shortleaf X loblolly pine hybrids to inoculation with fusiform rust, p. 113-118. Thirteenth South. For. Tree Improv. Conf. Proc., Raleigh, NC, 262 p.

Zak, Bratislav. 1955. Inheritance of resistance to littleleaf in shortleaf pine. USDA For. Serv. Res. Note SE-88, 2 p., Southeast. For. Exp. Stn., Asheville, NC.

REMOVAL OF COMPETITION BIAS FROM FOREST GENETICS EXPERIMENTS

D. T. Cooper and Robert B. Ferguson[1/]

Abstract.--Estimates of genetic gains and of juvenile-mature correlations in small-plot breeding experiments may be inflated because trees that grow rapidly early continue to be the largest trees, and trees that begin slowly usually stay small. A procedure which takes missing trees, relative sizes and distances between competing trees, and the intensity of competition into consideration was used to adjust diameter measurements in small-plot cottonwood clonal breeding experiments. The F ratio of clone to error mean square was increased and predicted genotypic gain was decreased.

Additional keywords: Cottonwood, genotypic gain.

In field experiments of planted trees, there normally are missing trees and trees considerably larger or smaller than their neighbors. As a result, the growing space available to a tree will differ from the space originally allotted. Trees with rapid early growth may appear better and trees with slow initial growth may appear poorer than their inherent potential. It is difficult for trees with poor early growth to catch up since they are suppressed by larger trees. Thus, the breeder may overestimate the genetic variability among experimental genotypes and predict greater genetic gains than are actually attainable. In addition, estimates of genetic correlations between measurements taken at different ages can be inflated, causing the breeder to believe that early selection is more effective than it really is. The problem is particularly serious in cottonwood, where a high incidence of missing and small trees resulting from the use of unrooted cuttings, rapid growth rate, and sensitivity to crowding cause competition bias to become important at an early age.

Partial solutions to the problem include: (1) reducing competition by wide spacing or early thinning, (2) causing competition to be as uniform as possible by testing only clones with similar growth potential (possible in advanced clonal tests) and by planting two or more cuttings per spot and thinning back soon after establishment to the best tree to reduce the number of missing spots and to improve uniformity of early growth, and (3) using data adjustment procedures which compensate for the effects of missing and suppressed trees. A combination of the above procedures should give the best results. This paper describes a data adjustment procedure and its application to diameter data in small-plot cottonwood clonal tests. It illustrates what happens when data are adjusted and may provide a starting point from which better procedures can be developed.

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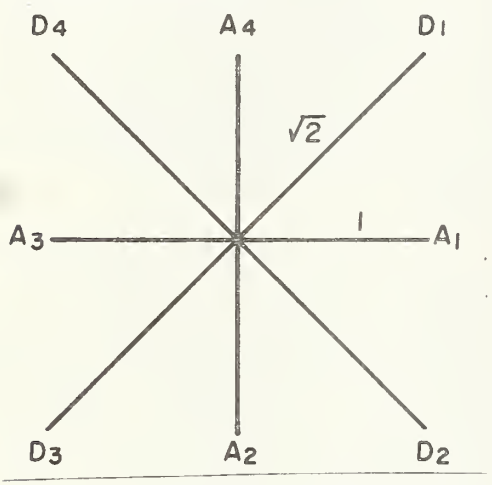
MATERIALS AND METHODS

Missing trees, relative sizes and distances between competing trees, and intensity of competition were taken into consideration in choosing an adjustment procedure which would tend to convert tree diameter to that which would have occurred if the tree utilized no more or less than its allotted space. Only the positions immediately surrounding the measurement tree were considered. It was arbitrarily assumed that the effect of one tree on another could be approximately described as the reciprocal of the square of the distance between them times the difference in their basal areas times a coefficient which would reflect the intensity of competition. Thus, with a square grid arrangement of trees, a diagonal neighbor would have only one-half as much effect as an adjacent neighbor of the same size. The coefficient would be zero if there is no competition among trees or if they compete evenly. It would increase as uneven competition develops. Proper adjustment would depend on an accurate estimate of this coefficient.

The adjustment computation for trees arranged in a square grid pattern was made as follows:



$$J = B - CE$$

where J = adjusted basal area
B = measured basal area
C = competition coefficient for the experiment
E = adjustment value =

$$\left(\sum_{i=1}^{4}(D_i - B)\right)/12 + \left(\sum_{i=1}^{4}(A_i - B)\right)/6$$

where D_i = measured basal area for the ith diagonal neighbor
A_i = measured basal area for the ith adjacent neighbor

Programs were developed in BASIC for a HP9830A computer[1/] to allow efficient computation of the adjustment. Data were divided into manageable arrays and stored on tape. Data were then converted to basal area per tree and an adjustment value computed for each tree. The unadjusted dbh, unadjusted basal area, and the adjustment value were then printed out. Values from missing and extremely small trees were deleted and identification, unadjusted basal area, and adjustment values were re-entered. Analyses of variance based on plot means and separate analyses of within plot variance were then performed repeatedly with various competition coefficients. The effects of adjustment on within-plot variance, replication x clone variance, clone variance, and F ratio of clone to error mean square for clones were examined.

[1/] The use of trade name is for the information and convenience of the reader. Such use does not constitute an official evaluation, conclusion, recommendation, endorsement, or approval of any product or service to the exclusion of others which may be suitable.

The adjustment routine was applied to four separate cottonwood clonal studies that represented a range of genotypes, plot sizes, planting sites, ages, and competition intensities.

Study 1 consisted of 12 random clones from a single full-sib family. There were 2 replications of 4-tree linear plots at 12 x 12 ft. spacing. Mean dbh was 5.6 in. at age 3 and 7.3 in. at age 5. Crowns were still relatively full at age 5 but it appeared to be time for thinning in order to keep the trees growing rapidly.

Study 2 consisted of from 2 to 4 random clones from each of 16 full-sib families produced by crossing 4 superior female parents with 4 superior male parents. Two replications of two-tree plots at 12 x 12 ft. spacing were used. Mean diameter was 6.2, 6.8, and 7.2 in. at ages 4, 5, and 6 respectively. The general appearance of the crowns indicated that considerable competition was occurring by age 4. Data adjustment procedures were applied and clones not present in both replications were then dropped from further analysis leaving 48 clones.

Study 3 originally consisted of 25 select clones, 4 replications, and 2-tree plots at 10 x 20 ft. spacing. Three cuttings were planted per planting spot and thinned to the one best tree in June of the first growing season. One tree per plot was removed at age 5 leaving the remaining trees at 20 x 20 ft. spacing. The trees averaged 6.5 inches dbh at age 4 and produced a consistent 0.8 in. annual diameter increment for the next 6 years, slowing down to less than 0.4 in. annual increment during the eleventh and twelfth years. The data adjustment procedure was applied to age 12 dbh. Clones missing in one or more replications were excluded from further analysis, leaving 19 clones. Because of wide initial spacing, use of multiple cuttings, early thinning, and inclusion of only good clones in the study, the appropriate coefficient of competition was expected to be small, although mean dbh was 12.0 in. and basal area was 89 sq. ft. per acre.

Study 4 consisted of a single clone (Stoneville 66) planted at 10 x 10 ft. spacing. Three blocks, each 10 trees by 10 trees, were chosen at random. The trees averaged 3.0 in. dbh at age 3 and 3.6 in. dbh at age 4, but because of insufficient late-season moisture in the Sharkey clay soil at this site, competition was probably already important by age 3. Adjustment values were computed on the interior 8 tree by 8 tree portion of each block. Analyses of variance of adjusted values were computed considering each block as being made up of 1-, 2-, and 4-tree plots.

Replication x clone variance dropped less rapidly than within-plot variance. Replication x clone variance was minimized when the competition coefficient was 0.4 at age 3 and 0.5 at age 5 in Study 1 (Fig. 2). In Study 2 it was minimized at 0.6, 0.7, and 0.6 for ages 4, 5, and 6 respectively. It was minimized at 0.6 in Study 3. In Study 4 it was minimized at 0.8, 0.7, and 0.6 at age 3 and at 0.7, 0.6, and 0.5 for 1-,2-,and 4-tree plots at ages 3 and 4 respectively.

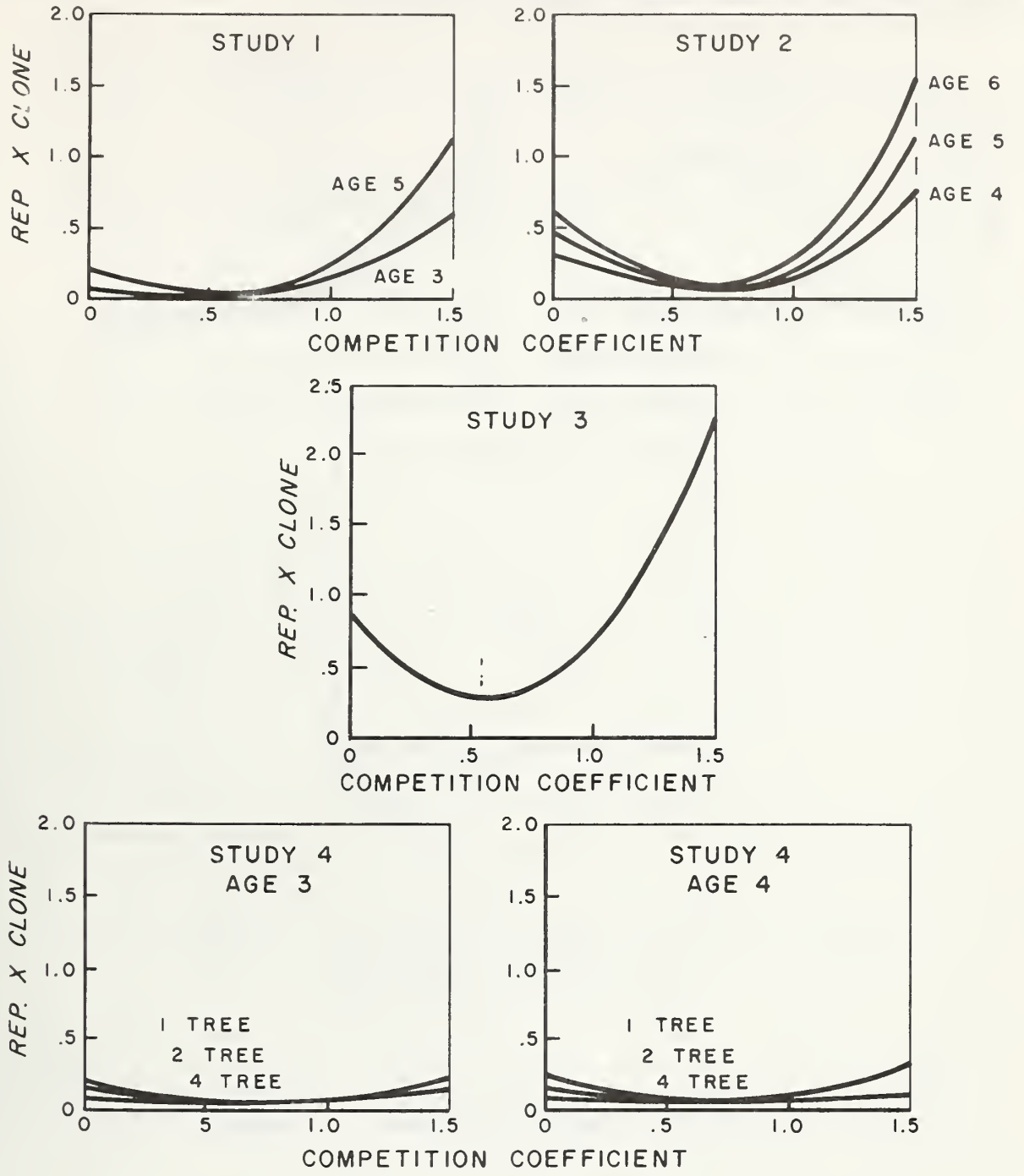
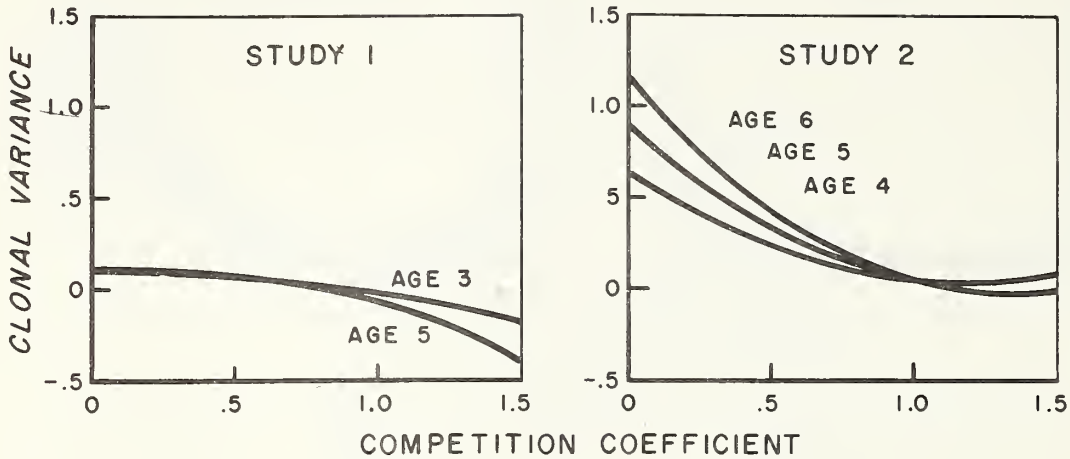


Figure 2.--Differences in replication x clone variance as competition coefficient is changed in Studies 1, 2, 3, and 4.

The pattern of change in the clone component of variance differed considerably among studies. As a result, the patterns of change for genotypic gain and the F ratio of clone to error mean square differed.



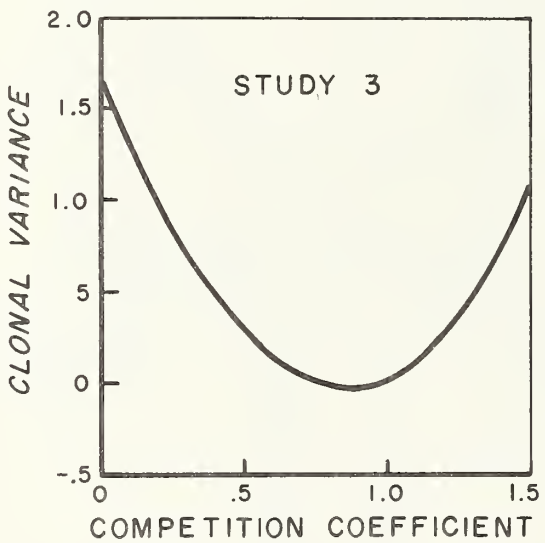


Figure 3.--Differences in clonal variance as competition coefficient changed in Studies 1, 2, and 3.

The F ratio increased initially and then dropped (Fig. 4). The competition coefficient for maximum F in Study 1 was 0.4 at age 3 and increased to 0.5 at age 5. In Study 2, F was maximized when the competition coefficient was 0.5 for ages 4, 5, and 6. F was maximized in Study 3 when the competition coefficient was only 0.1.

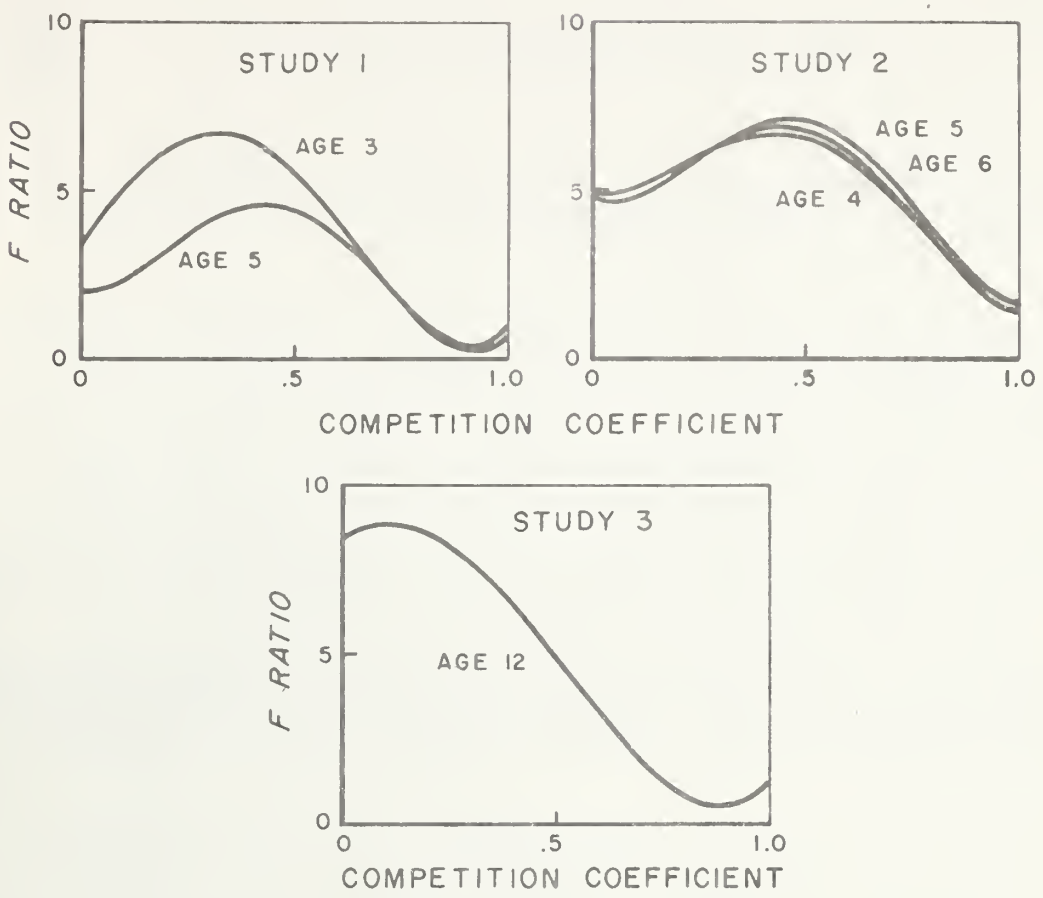


Figure 4.--Differences in F ratio of clone to error mean square as competition coefficient changed in Studies 1, 2, and 3.

The competition coefficient required to maximize the F ratio appeared to correspond with the apparent amount of competition observed in the various studies much better than the competition coefficient required to minimize within-plot or replication x clone variance. Maximizing F usually decreased predicted gain. In Study 1, predicted gain for data adjusted to maximum F was 89 and 104 percent of that of unadjusted data at age 3 and age 5 respectively. In Study 2, predicted gain for data adjusted to maximum F was approximately 65 percent of that of unadjusted data at each age. In Study 3, predicted gain for data adjusted to maximum F was 88 percent of that for unadjusted data.

DISCUSSION

The adjustment procedure takes missing trees, relative sizes and distances between competing trees, and intensity of competition into consideration to adjust diameters of large trees with little competition downward and diameters of small trees with intense competition upward. It shifts part of the area-related growth to missing positions which then are omitted from further data analysis. Values for trees which fail to take advantage of extra growing space are reduced and values for trees that grow well despite competition are increased. Thus, the results express something slightly different from the ability of the trees to grow in uniform, genotypically pure stands, but the error should favor clones capable of utilizing all available space.

Several improvements could possibly be made in the adjustment procedure. The effect of direction of the various neighbors on tree growth was ignored, which may not be accurate for crown competition but should be acceptable for below-ground competition. Trees more than one position removed from the tree for which adjustment was made could be taken into account. The reciprocal of the square of the distances between trees in the adjustment formula was chosen arbitrarily, and a greater increase in the F ratio might occur if a value different than the square was used. The adjustment procedure considered the relative sizes of the trees at a single time and it might be better to use the relative increase in size of the trees during the period just before adjustment.

The adjustment procedure is not suitable for removing microsite or soil gradient differences. It should be valuable on relatively uniform sites with fairly homogeneous material where missing and small trees result in unequal growing space per tree.

DEVELOPMENT AND POTENTIAL OF

A LONGLEAF PINE SEEDLING SEED ORCHARD

D. L. Rockwood and H. R. Kok[1]

Abstract. -- A longleaf pine progeny test designed for conversion to a seedling seed orchard was established near Gainesville, Florida, in 1969, with wind-pollinated progenies of 65 ortets of superior form and vigor selected in southeast Georgia, north Florida, and south Alabama and Mississippi. A high degree of variation among progenies was noted for survival, height initiation, height, and volume. Height initiation was positively correlated with growth and survival, but survival was not significantly associated with growth. The orchard was reduced to 152 trees per acre by within-family roguing at age 4 and family roguing and subsequent thinning within selected families at age 7. A total of 53 progenies were retained, but 34 progenies constituted most of the orchard. Forty-four percent of the 34 were from south Alabama and west Florida. Suggested gains from retaining 34 progenies were 41 percent for height initiation, a large increment for survival, and 61 percent for volume, but due to the index selection practiced, the gains will be lower. The orchard has been fertilized to promote flowering, and a number of progenies have conelets.

Additional keywords: Pinus palustris, selection, tree improvement.

Longleaf pine (Pinus palustris Mill.) currently is a much less important component of Southern forests than it was in colonial times (Croker and Boyer 1975). The species has not been widely employed for artificial regeneration due to its low planting survival, susceptibility to brown spot disease, and long grass stage. However, inherent properties of longleaf such as excellent form, high specific gravity (Wahlgren and Schumann 1975), and good naval stores production plus its potential as a source of fusiform rust resistance (Dinus 1974) favor wider utilization. Recent improvements in cultural practices and results of breeding efforts (Snyder 1973; Goddard et al 1973; Goddard et al 1976) further inhance greater exploitation. One of the efforts of the Cooperative Forest Genetics Research Program at the University of Florida with longleaf pine, the development of a progeny test-seedling seed orchard, is discussed.

METHODS

Seed were collected from 67 ortets, superior phenotypes for vigor and

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form, located from southeast Georgia, north Florida, and south Alabama and Mississippi, and established in the St. Regis Paper Company nursery at Lee, Florida, in 1968. Five tests were hand-planted in January, 1969: 65 progenies in Alachua County, Florida, and lesser numbers of progenies in Wayne County, Georgia, Nassau County, Florida, and Baldwin and Escambia Counties, Alabama. The Baldwin and Escambia plantings were failures.

The Alachua County progeny test-seed orchard was established on a typical flatwoods site that had previously supported longleaf; the soil was classified as a Pomona fine sand, somewhat poorly drained. Thorough site preparation and disking preceded planting. On the 2.7 acres, twelve replications were employed using progeny row plots of 5 trees. Spacing was 10 feet between rows and 3 feet within rows. Subsequent maintenance involved annual disking.

The test was measured periodically starting the first year. Percent survival and percent of trees initiating height growth were determined at ages 1 and 2. These two measurements plus total height of all living trees were taken at age 3. At age 4, survival and total height were remeasured. After the fourth year measurements, each plot was thinned to a maximum of the best two trees. Height and dbh of the residual trees were determined at age 7, and tree volumes were calculated.

Analyses of variance performed were least square analyses due to the failure of some progenies to occur in each replication. Progenies represented in six or less replications were deleted from analyses. Analyses were conducted using plot means, and in the case of height and volume, within-plot variation was also calculated. Variance components were derived for computation of heritabilities. Harmonic mean numbers of trees per plot were calculated in order to combine within and between plot analyses.

Individual heritabilities for height and volume were calculated by standard formulae employing variance components. For survival and height initiation, individual heritabilities were derived by the threshold approach (see example in Goddard and Arnold 1966). Progeny mean heritabilities were calculated for all traits. Standard errors were associated with all heritability estimates (Osborne and Paterson 1952). Predicted gains, assuming original selection of 1 in 1000 parent trees and retention of 51 percent of the families and 7 percent of the individuals in selected families were obtained (Shelbourne 1969).

Progeny roguing was conducted in April, 1976, by an index system that weighted survival, height initiation, and volume equally. Survival and height initiation assessments were taken from as many of the three surviving tests as possible; volume data were obtained only from the orchard test. Progenies were classified by the number of traits for which each exceeded the average. Ten progenies below average for all traits were eliminated. Nineteen progenies better than average for any one trait were subjected to within-family roguing of 50 percent, 23 progenies exceeding the average for two traits to 33 percent, and 11 progenies above average for all traits to 25 percent.

RESULTS AND DISCUSSION

Survival, although only 45 percent after the first year, decreased little through age 4 (Table 1), and relationships among progeny means for survival during the four years were extremely strong (Table 2). The percentage of trees out of the grass stage was 27 after one year and averaged 67 after three years. Progeny performance for height initiation at age 3 was not correlated with the first year's, but the age 2 evaluations were. Average tree heights after three years were low but were well correlated with heights at ages 4 and 7. Fourth year height was associated equally well with seventh year height and volume, an indication that the removal of the poor phenotypes at age 4 did not bias subsequent progeny evaluations for growth.

Progeny assessment for survival, height initiation, and growth rate can apparently be made by age 3, at least on average or better sites when control of competing vegetation is practiced. Survival evaluations could be made as early as the first year and height initiation by second year, but three years are suggested for an appraisal including growth. Similar recommendations have been made for longleaf pine in South Carolina (Schoenike and Williams 1975).

Relationships among the traits indicate that progenies having high survival, early height initiation, and fast growth can be obtained. Third year height initiation was highly correlated with third year survival and height. Survival, however, was positively but not significantly associated with height at either age 3, 4, or 7 (Table 2); a similar conclusion has been reported from another study (Schoenike and Williams 1975). Consequently, progenies with above average height or volume coupled with good survival and a short grass stage can be obtained only by screening a large number of families. In this orchard, the 20 "best" progenies were 16, 24, and 19 percent better, respectively, for height initiation, survival, and height, but not all progenies exceeded the average for each trait (Table 3).

Brown spot was not a problem in the orchard, and no observations on incidence were taken. Consequently, the better progenies cannot be directly characterized from brown spot resistance. With their strong tendency to initiate height growth early and for fast growth, the progeny are likely to possess some resistance since the two traits have been associated with resistance (Snyder and Derr 1972; Schoenike and Williams 1975).

Thirty-four progenies constituted the bulk of the orchard at age 7. Of these, 44 percent were from the "optimum" seed collection area for longleaf (Wells and Wakeley 1970). Twenty-five percent of the progenies heavily or completely rogued from the orchard were from the "optimum" area. Thus, the reported superiority of the western Gulf sources is somewhat in evidence.

Performance of the eastern selections, which represent geographic sources not included in the Southwide Pine Seed Source Study, indicate that southeast Georgia and north Florida longleaf should be included in

Table 1.--Performance of longleaf pine progenies by trait and age.

Trait		Age 1	2	3	4	7
Survival (%)	Mean	45	44	42	41	
	Range	18-82	18-78	17-78	12-80	
Ht. Initiation (%)	Mean	27	41	67		
	Range	0-74	6-75	25-100		
Total Ht. (ft)	Mean			1.1	3.7	17.2
	Range			.5-2.1	1.1-5.5	14.9-20.0
Volume/Tree (ft^3)	Mean					.352
	Range					.246-.513

Table 2.--Correlations among longleaf progeny means for survival, height initiation, height and volume at various ages.

Trait	Age:	Survival 2	Survival 3	Survival 4	Height Initiation 2	Height Initiation 3	Height 4	Height 7
Survival	1	.99**	.97**	.96**				
	2		.99**	.97**				
	3			.98**		.53**		
	4					.41**	.20	.17
Height Initiation	1				.60**	.25		
	2					.53**		
	3						.54**	.50**
Height	3			.24		.66**	.93**	.74**
	4							.79**
	7							
Volume	7			.06		.39*	.74**	.92**

* and ** - significant at the 5 and 1 percent levels, respectively.

Table 3.--Performance of the 20 "best" longleaf progenies for height initiation, survival, height, and volume.

Progeny	Parent Location	3rd Yr. Ht. Init.	4th Yr. Survival	4th Yr. Height	7th Yr. Vol./Tree
	(County)	(%)	(%)	(ft)	(ft^3)
102-61	Alachua, FL	74	57	4.1	.354
111-61	Alachua, FL	82	37	4.5	.451
59-63	Wayne, GA	75	45	4.0	.341
122-63	Camden, GA	68	38	4.5	.439
135-63	Santa Rosa, FL	77	66	3.7	.353
7-65	Escambia, FL	78	36	4.6	.358
12-65	Escambia, AL	66	54	4.3	.301
13-65	Escambia, AL	88	64	4.9	.326
15-65	Santa Rosa, FL	66	63	3.7	.391
24-65	Santa Rosa, FL	72	68	4.6	.375
50-65	Bacon, GA	100	43	4.1	.326
55-65	Bacon, GA	70	56	3.6	.379
58-65	Bacon, GA	81	55	5.2	.462
8-66	Hamilton, FL	90	47	5.5	.478
9-66	Hamilton, FL	77	52	5.4	.513
10-66	Hamilton, FL	83	49	5.2	.433
13-66	Hamilton, FL	84	47	4.0	.392
37-66	Glynn, GA	72	38	4.2	.491
61-67	Mobile, AL	65	47	3.4	.341
79-67	Alachua, FL	82	58	4.1	.447
Average		78	51	4.4	.398
Test Average		67	41	3.7	.352

a breeding program. The basis for our progeny evaluations, plantings in Wayne County, Georgia, and Alachua and Nassau Counties, Florida, may of course favor the eastern selections. Testing of these sources further west is needed for height, volume, height initiation, and brown spot resistance; survival relative to western sources will be lower (Goddard et al 1971).

Variation among progenies in the orchard was noted for each trait (Table 4). A high degree of variation among replications was attributed to one tier of replications being adjacent to a deep drainage ditch. Instability of progeny performance across replications was evident for fourth year height but not seventh year volume.

The variation in height initiation at age 3 was the least heritable of four traits, but a meaningful reduction in the length of the grass stage is predicted (Table 5). Heritability of survival was high, and a large gain was derived.

Table 4.--Analyses of variance for height initiation at age 3, survival and height at age 4, and volume at age 7.

Source	Height Initiation d.f.	MS	F	Survival d.f.	MS	F
Reps	11	6,322	4.85**	11	6,477	10.59**
Progenies	56	2,300	1.77**	56	2,737	4.47**
Error	530	1.303		582	612	

Source	Height d.f.	MS	F	Volume d.f.	MS	F
Reps	11	18.36	8.30**	11	.1455	11.05**
Progenies	49	6.05	2.73**	43	.0450	3.42**
Error	413	2.21	1.52**	351	.0132	1.24
Within Plots	730	1.46		182	.0107	

**significant at the 1 percent level.

Table 5.--Individual and progeny mean heritabilities, their associated standard errors, and expected gains for height initiation at age 3, survival and height at age 4, and volume at age 7.

Trait	h^2_I	h^2_x	Gain Unit	Gain Percent
Height Initiation	.17	.41 ± .12	28%	41
Survival	.33	.73 ± .17	54%	131
Height	.32 ± .09	.58 ± .09	2.6 ft	70
Volume	.51 ± .14	.62 ± .09	.216 ft^3	61

The progeny mean heritability of fourth year height was comparable to other reported estimates for height (Snyder 1973; Snyder and Derr 1972) and similar to the heritability of seventh year volume. The magnitudes of gains expected for both growth traits were also alike. A considerable improvement in volume per unit area is anticipated, however, due to the composite selection for survival and growth, as was observed for longleaf in Mississippi (Snyder 1973).

Two reasons for expecting less gain from the orchard than the above cited predictions are the index selection used for progeny roguing and the statistical analysis of data from only the orchard test. The selection of progenies was based on their evaluation equally for survival, height initiation, and growth; only a few of the 34 progenies assumed to be retained for the production orchard excel in all three traits. Consequently, actual gains from these progenies will be less.

The progeny evaluations for survival and height initiation were also based on progeny evaluations over as many as three tests. An analysis of composite test data, incorporating genotype by environment effects, would give more applicable gain estimates for the two traits. Even considering that the expected gains given will be lower, the potential for improving each trait seems good.

Fertilization of the orchard during the eighth growing season following roguing to 152 trees per acre promoted conelet production; 95 of 406 trees had conelets at age 9. Nitrogen and nitrogen plus phosphorous applications increased the proportion of progenies and trees flowering in addition to the number of conelets per tree. Phosphorous alone produced little response (Table 6).

Table 6.--Conelet production in the longleaf orchard following fertilization.

Variation Among Progenies		Influence of Fertilizer			
			Percent Flowering		Conelets Per
Conelets Per Tree	Number of Progenies	Fertilizer	Progenies	Trees	Flowering Tree
0	21	Check	20	11	2.6
1-5	30	N	54	31	10.3
6-10	1	P	23	15	2.4
11-15	1	NP	46	32	7.3
	53				

Variation among progenies was evident. Some 60 percent of the progenies had conelets, but only two progenies had sizeable numbers of conelets per tree. One progeny had 88% of its trees flowering, one of which had 32 conelets. Correlations of flowering with other traits were nonsignificant although a slight inverse relationship with seventh year growth existed.

These flowering patterns indicate that longleaf seedling seed orchards can be a relatively quick source of seed. Flowering has been noted by age 9, and certain fertilizers seem to promote flowering.

Alternatively, the number of progenies and trees not yet flowering and the scarcity of conelets per tree suggest another emphasis in longleaf pine selection. The parents of these orchard progenies were outstanding phenotypes for vigor and form; they were not selected for cone productivity. Evidence suggests that parental selection is not highly productive and that progeny evaluation is needed for best identification of good surviving, fast growing trees (Snyder 1973; Goddard et al 1973). Parental consideration for cone productivity should be emphasized however in order to offset longleaf pine's notoriously bad cone production and an apparently slight tendency for fast growing trees to have lower flowering rates.

CONCLUSIONS

A seedling seed orchard appears to be a feasible method of producing longleaf pine planting stock suitable for artificial regeneration. Seed production can be achieved by age 10 with resulting improvement in survival, height initiation, and growth rate. Parental selection criteria should include cone productivity,and sufficient parents should be employed to derive progenies with good overall performance. Appropriate maintenance and fertilization of the orchard are required.

LITERATURE CITED

Croker, T. D., Jr. and W. D. Boyer. 1975. Regenerating longleaf pine naturally. USDA FS Research Paper SO-105. 21p.

Dinus, R. J. 1974. Knowledge about natural ecosystems as a guide to disease control in managed forests. Proc. Amer. Phytopath. Soc. 1: 184-90.

Goddard, R. E. and J. T. Arnold. 1966. Screening select slash pines for resistance to fusiform rust by artificial inoculation. In Breeding Pest Resistant Trees. p. 431-5.

Goddard, R. E., C. A. Hollis, III, H. R. Kok, D. L. Rockwood, and R. K. Strickland. 1973. Cooperative forest genetics research program. 15th progress report. Univ. Fla. Sch. of For. Res. and Conserv. Res. Rept. No. 21. 19p.

Goddard, R. E., D. L. Rockwood, C. A. Hollis, III, H. R. Kok, and J. A. Hendrickson. 1976. Cooperative forest genetics research program. 18th progress report. Univ. Fla. Sch. of For. Res. and Conserv. Res. Rept. No. 25. 20p.

Goddard, R. E., R. K. Strickland, and H. R. Kok. 1971. Cooperative forest genetics research program. 13th progress report. Univ. Fla. Sch. of For. Res. and Conserv. Res. Rept. No. 19. 20p.

Osborne, R. and W. S. B. Paterson. 1952. On the sampling variance of heritability estimates derived from variance analyses. Proc. Roy. Soc. Edinb. B. 64: 456-61.

Schoenike, R. E. and J. G. Williams, Jr. 1975. Ten-year results of an open-pollinated progeny test of longleaf pine in South Carolina. Proc. 13th South. For. Tree Imp. Conf. p. 97-104.

Shelbourne, C. J. A. 1969. Tree breeding methods. New Zealand Forest Service Tech. Paper. No. 55. 43p.

Snyder, E. B. 1973. Fifteen-year gains from parental and early family selection in longleaf pine. Proc. 12th South. For. Tree Imp. Conf. p. 46-9.

Snyder, E. B. and H. J. Derr. 1972. Breeding longleaf pines for resistance to brown spot needle blight. Phytopathology 62(3): 325-9.

Wahlgren, H. E. and D. R. Schumann. 1975. Properties of major southern pines. Part I - Wood density survey. USDA FS Research Paper FPL 176. 76p.

Wells, O. O. and P. C. Wakely. 1970. Variation in longleaf pine from several geographic sources. For. Sci. 16: 28-42.

SESSION II - SEED ORCHARD TECHNOLOGY

MODERATOR: LEE DRAPER, JR.

SEED ORCHARD PEST MANAGEMENT STRATEGIES

Harry O. Yates, III[1]

Abstract.--Pests in seed orchards have traditionally been controlled by total coverage of the seed crop with pesticides. A concept central to pest management, however, is to accept pest populations and pest-caused losses within calculated limits. Although insecticides are the only materials presently available to control pests in seed orchards, the orchard manager should determine when these controls are necessary, set control priorities, and evaluate their effectiveness in managing insect populations. Product inventory should be an integral part of seed orchard management. Without such an inventory, the size of the crop and annual losses cannot be determined. When insecticides are used more concern should be shown for minimizing the amounts applied. Selective tree treatment based on crop size should be considered as a viable alternative to total orchard treatment.

The concept of pest management dictates toleration of minor damage by pests (Metcalf and Luckmann 1975). In other words, not all pest damage is intolerable in a forest or a seed orchard. Rabb (1972) defines pest management as "...the intelligent selection and use of pest-control actions that will ensure favorable economic, ecological, and sociological consequences."

A key term in this definition is the word, "favorable." So often we see the goal of seed orchard protection as insuring maximum production of viable seed. Surely this goal is within the stated objectives of any seed orchard manager. However, in pursuit of this goal we sometimes fail to recognize that there are other factors to consider. If pesticides are excluded, present knowledge suggests the orchard will sustain enormous seed losses. But if pesticide is relied on too heavily, the ecological and economic consequences may be unacceptable. Therefore, pest management must be considered as a balanced approach to product protection. In a business sense it is a balancing of credits and debits to produce a favorable return on investment.

My report is restricted to insects that reduce viable seed production in the seed orchard. However, many of the ideas presented can be applied to other seed orchard pests like diseases, birds, or rodents.

At the conclusion of the 12th Southern Forest Tree Improvement Conference in Baton Rouge, La., in 1973, an *ad hoc* committee reported the results of a questionnaire survey in which seed orchard managers were asked about existing or anticipated problems and the urgency of their solution (Dinus *et al.* 1973). This report stressed that the "most pressing concern in southern forest tree improvement is clearly the severe, continuing reduction in cone and seed crops caused by insects." Since that time, substantial progress has been made toward solving this problem.

[1]Principal Research Entomologist, Southeastern Forest Experiment Station, USDA Forest Service, Athens, Ga.

Perhaps the most important initiative was the formation of the Southern Seed Orchard Pest Committee (SSOPC) to coordinate insecticide testing, evaluate control effectiveness, and follow through to insecticide registration by the Environmental Protection Agency. This Committee's work resulted in two important accomplishments. First, in 1974 the Committee succeeded in getting registration for Guthion® (azinphosmethyl) extended from control of coneworms (Dioryctria spp.) in slash pine seed orchards only to control of coneworms in all southern pine seed orchards. However, this insecticide has a relatively high mammalian toxicity and must be sprayed four or more times during the growing season to be effective. Both of these characteristics are undesirable. Therefore, as a second goal, the Committee set out to find an effective substitute for Guthion®.

A two-year regional cooperative study extending from Pennsylvania to Texas culminated in the fall of 1976 with the registration of granular Furadan® (carbofuran) by EPA for control of seedbugs (Leptoglossus corculus, Tetyra bipunctata), coneworms, coneborers (Eucosma spp.), and cone beetles (Conophthorus spp.) in southern pine seed orchards. A great number of seed orchard managers have begun using this material in their insect control programs during the 1977 season.

Currently, the two insecticides, Guthion® and Furadan®, are the only compounds registered for protecting cone crops in southern seed orchards from insects. Unfortunately, we have no other proven control methods. Therefore, when considering pest management of seed orchard insects we have few alternatives. The manager can, however, determine when these controls are necessary, set control priorities, and evaluate effectiveness in managing pest populations. The remainder of this paper will be devoted to these points.

Product Inventory

It is not only important to know what pests are causing losses and when; it is equally important to know the volume of the crop needing protection.

When I look at the pest monitoring programs presently being conducted in seed orchards, I am reminded of the story about a tool manufacturing company. To control the stealing of tools by employees, the company placed a guard at the gate to search everyone's lunch box as he left at the end of the work day. Each day one particular employee showed up at the gate pushing a wheelbarrow covered with a white cloth. Dutifully each day, the guard checked the man's lunch box and looked under the white cloth covering the wheelbarrow for stolen tools. This continued for weeks and months and yielded no stolen tools. However, the company continued to experience significant losses. It was not until some enterprising young executive decided to institute a product inventory control system that the company found out that their major losses stemmed from one of their employees stealing wheelbarrows.

A lesson to be learned from this story is that to justify controlling pests in seed orchards, we must know the quantity of the product to be protected, when losses are sustained, and finally the causes of these losses. In short, some form of product inventory or accountability must be developed to extend throughout the term of seed development (from flower bud formation to seed harvest). Such a product inventory or accountability system can be achieved with life tables.

A number of workers have completed life tables for seed crops of pines. Such tables have been produced for slash pine in Florida (DeBarr and Barber 1975), and for shortleaf (Ebel and Yates 1974) and loblolly pines (Yates and Ebel, In Press) in the Georgia Piedmont. In these studies, the sequence of insect-caused damage and mortality was recorded for 2 or more years.

Figure 1 shows a life table developed for shortleaf pine. The lines on the graph show numbers of surviving conelets and cones over the 2-year cone development period. During life table development, each mortality factor and its importance in limiting the potential seed crop can be isolated. The relative importance of each destructive agent and its attack period can be determined. With this information, the manager can determine when controls are best applied.

Because of their small size, newly emerging female flowers are the most difficult seed producing structure to inventory; they appear during the late winter or early spring. Despite the difficulties, these structures must be counted because their numbers form the baseline for the continuing inventory. Periodic flower, conelet, and cone inventories on the same trees should be conducted throughout the 2-year period of seed development. Then the harvested cones should be analyzed as described by Bramlett et al. (In Press). With this data, product life tables can be developed to indicate the size of initial crop, when losses occur, and the agent(s) responsible for these losses.

There seem to be two obstacles to the widespread use of such a product inventory control system. One is the reluctance of managers to sacrifice the product of selected check trees from which insect controls are withheld, and the second is the apparent unwillingness of managers to spend the time to make initial and subsequent counts. These concerns may seem reasonable, but a continuing product inventory is needed to rationally plan for pest control. Without such a "Judas plot" and periodic inventories, your pest control practices may be protecting a crop of unknown size from imaginary dangers. Until such an inventory system is instituted, you cannot establish the relative importance of various attacking agents. More importantly, you cannot set meaningful priorities on control procedures and determine their effectiveness.

In the seed orchard we are concerned with protecting the developing seed crop for roughly 2½ years. During this developmental period, the crop is subjected to an array of pests (Ebel et al. 1975), primarily insects, which may attack a specific fruiting structure such as flowers, conelets, and cones for varying periods of time. For instance, the Nantucket pine tip moth, Rhyacionia frustrana (Comstock), damages or kills female buds and flowers of shortleaf and loblolly pines during April through June of the first growing season (Fig. 2). In contrast, the blister coneworm, Dioryctria clarioralis (Walker), which attacks all species of southern pines, attacks shoots, buds, conelets, and cones throughout the entire two growing seasons (Feb.-Nov.) (Fig. 3).

Effective control of each of these insect pests provides a different return on the control investment. In this example, control of the Nantucket pine tip moth could be expected to increase yield of harvested cones by about 15 percent (dotted line) (Fig. 2), and treatment would be required for only 2½ months during the first season. The increased yield due to effective blister coneworm control would be only about 5 percent and would require a 4½ month

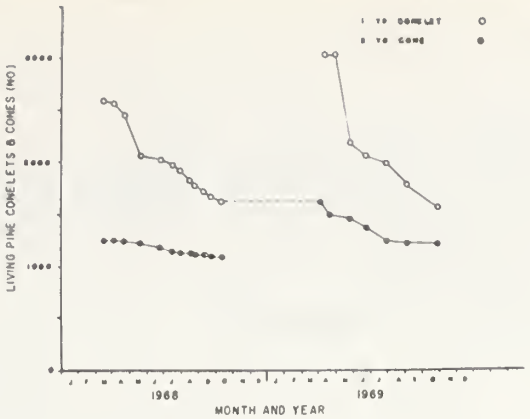

Figure 1.--Life table of shortleaf pine seed producing structures. Circles indicate dates that data was taken. First-year conelets are open circles; second-year cones are black dots.

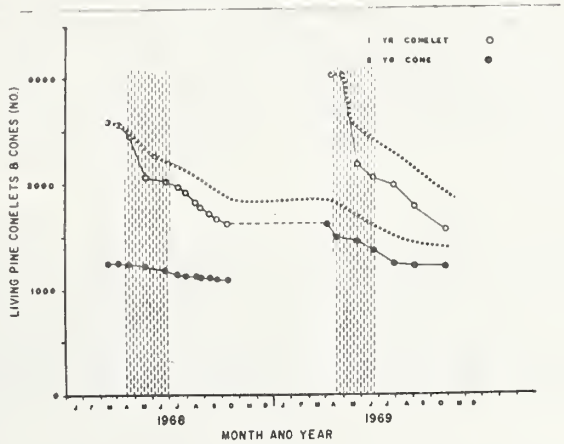

Figure 2.--Life table of shortleaf pine seed producing structures showing attack period of the Nantucket pine tip moth (shaded area). The dotted line indicates the expected increased yield of harvested cones if the Nantucket pine tip moth were controlled.

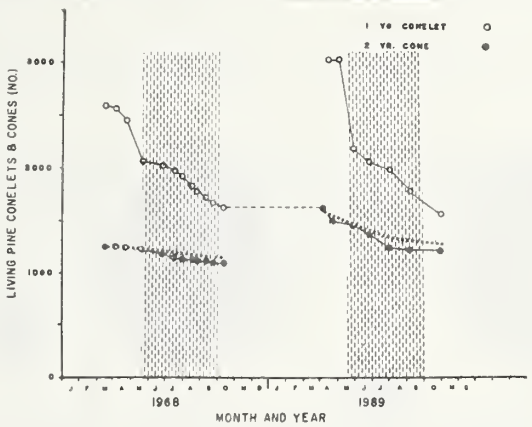

Figure 3.--Life table of shortleaf pine seed producing structures showing attack period of the blister coneworm (shaded area). The dotted line indicates the expected increased yield of harvested cones if the blister coneworm were controlled.

commitment for two seasons (dotted line) (Fig. 3). It is obvious which insect is the more important to control. These examples are for only 2 of the 22 known insect species or species complexes that the manager is confronted with in southern pine seed orchards.

Cost-Benefit Ratio of Product Protection

Since the registration of Furadan® for seed orchard use by the Environmental Protection Agency, a number of companies have decided to apply this granular insecticide once a year. While this treatment is highly effective, our present methods of application are both wasteful and environmentally unsound.

When insecticide formulations are sprayed, the operator usually can direct measured amounts of spray to cover tree crowns of various sizes. He can also terminate the spray discharge where missing trees create open areas in the seed orchard. Even with these capabilities, insecticide spray methods are not particularly noted for their efficiency. Most present mechanized methods of applying granular Furadan® in seed orchards have neither of these capabilities.

Figure 4 represents a series of trees ranging from 2 to 8 inches in diameter which might be found growing in a seed orchard. In applying granular Furadan®, the registration specifies 4-8 ounces of material per inch of tree diameter. Using an 8 oz. rate, an 8-inch tree should receive 64 ounces; a 6-inch tree, 48 ounces; and a 2-inch tree, 16 ounces of Furadan®. However, since most present mechanical systems do not allow the application rate to be varied while they are in operation, some managers would set the application rate for the 8-inch trees or at 64 ounces per tree. After all, the largest trees are usually the biggest cone producers and the treatment rate must be established accordingly. That means that one-half of the trees receive more insecticide than called for in the EPA approved registration (the 6-inch tree receives 10.6 ounces/inch of diameter and the 2-inch tree receives 32 ounces/inch of diameter). This is not only wasteful but down right illegal!

Figure 4.--Simulation of four seed orchard trees of varying diameters and cone crops.

Figure 5 shows a hypothetical seed orchard with trees 2, 6, and 8 inches in diameter. Missing trees are indicated by circles with a crossbar. Here again, let us assume we have decided upon a rate of 8 ounces per inch of tree diameter. Treatment of this complete plot would require 72 pounds of insecticide using a mechanized applicator. If, however, flow cutoffs were installed on the applicator, we could reduce this quantity to 60 pounds of insecticide, a 17 percent reduction (Fig. 6). A further refinement incorporating both flow cutoff and flow rate control would result in delivery of only 37 pounds of insecticide, a 49 percent reduction (Fig. 7). This last treatment would not only give adequate control based on research but would be in keeping with label registration requirements.

Selective Product Protection

Treatment based on tree size alone can be quite wasteful. As pointed out by DeBarr in 1971, it costs as much to protect a tree with half a bushel of cones as it does to protect one of equal size with four bushels. Therefore, it is important to consider the cost-benefit ratio of product protection. Figure 4 shows four trees with varying diameters and cone crops. It should be obvious that on the basis of product protection treatment priorities would be: (1) the 8-inch tree on the left with 28 cones, (2) the 6-inch tree with 11 cones, (3) the 2-inch tree with 5 cones, and (4) the 8-inch tree with only 5 cones.

Alan Lakein is a time planning and life goals consultant. In his book, "How to Get Control of Your Time and Your Life," he tells people to think more about what they do (Lakein 1973). He bills himself not as an efficiency expert but rather an effectiveness expert. Effectiveness, he says, is achieved by selecting the best task to do from all the possibilities available and then doing it the best way.

In all planning, we make lists, mental or written, and set priorities on the tasks to be accomplished based on what is important to us now. Mr. Lakein believes that if all the items on your list are arranged in order of value, 80 percent of the value would come from only 20 percent of the items. He calls this the 80/20 rule.

I would like to enlarge upon this rule by relating this principle to seed orchard pest management.

At the 1976 meeting of the Southern Seed Orchard Pest Committee in Atlanta, Ga., Gary DeBarr suggested that if managers concentrated their control programs on the clones that are good cone producers and on the trees that are most susceptible to insect attack, it might be possible to protect 90 percent of the seed crop by treating only 10 percent of the trees.[2] I have called this idea the "D 90/10 rule"--"D" for DeBarr.

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[2]Minutes of the Southern Seed Orchard Pest Committee Meeting, Atlanta, Ga., November 23, 1976.

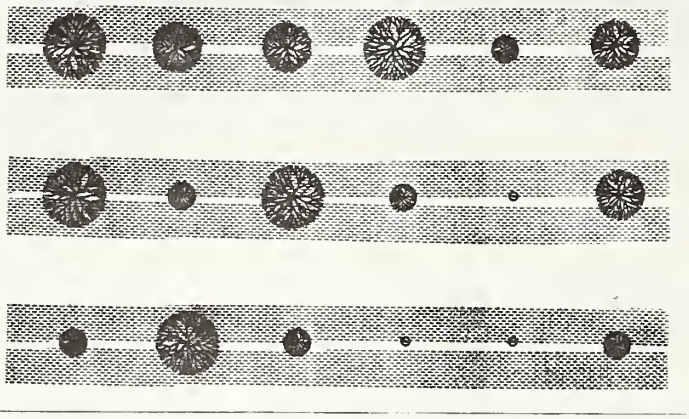

Figure 5.--Simulation of 18 seed orchard trees of varying diameters (2", 4", 6"). Open circle with crossbar indicates a tree location where tree is missing. Shading along tree rows shows distribution of granular insecticide by mechanical application.

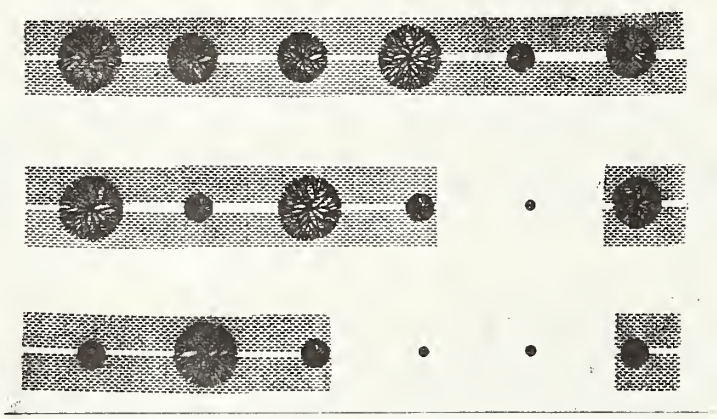

Figure 6.--Simulated seed orchard showing distribution (shaded area) of granular insecticide delivered by mechanical applicator with flow cutoff control.

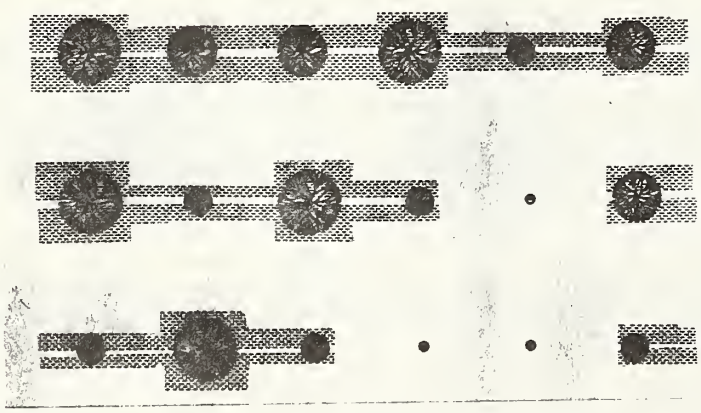

Figure 7.--Simulated seed orchard showing distribution (shaded area) of granular insecticide delivered by mechanical applicator with both flow cutoff and flow rate control.

In 1976 a fertilizer experiment was installed in a 12-acre block of shortleaf pine seed orchard trees at the Beech Creek Seed Orchard at Murphy, North Carolina.[3] This block contains 22 rows with 53 tree locations in each row. Total tree locations are 1166. Fifty clones are systematically replicated throughout this seed orchard. The cones on each tree were completely inventoried.

The seed orchard is pictorially reproduced in Figure 8. Trees are spaced at 15-foot intervals and rows are 30 feet apart. Each living tree is represented by a dark circle, and locations where a tree is missing are blank. Trees are in their 10th growing season.

In this discussion, it is assumed that Furadan® 10% granules will be applied at the registered rate of 8 ounces per inch of tree diameter. However, nearly all the same principles I present will apply to the use of any other registered chemical controls.

Treatment of this entire seed orchard block with granular Furadan® using a mechanized applicator will require about 3500 pounds of insecticide. That is: maximum tree diameter of 6 inches x 8 ozs. x 1166 tree locations = 3498 lbs. Since there is no cutoff on the delivery system of most existing mechanized applicators, all 1166 tree locations would be treated; since the study block contains only 875 living trees (trees are at 75 percent of the tree locations), 25 percent of the insecticide will be wasted during application. The amount applied could be reduced from 3498 lbs. to 2625 lbs. by putting a cutoff on the delivery system.

A more significant savings in insecticide could be made if the application technique could be regulated to account for differences in tree diameters as well as missing trees. The total tree diameter of the 875 trees in the seed orchard block is 3,290 inches (average tree diameter = 3.76"). Therefore, adequate protection of all trees would require only 1645 pounds of insecticide, and the savings would be 53 percent (1645 lbs. versus 3498 lbs.). Only by individual tree treatment is such a savings presently possible.

Up to this point we have considered treatment of all trees in the seed orchard. Now let's see what happens if we treat only the trees on which 90 percent of the cones are present.

When the 50 clones in the orchard are ranked according to numbers of cones present, it can be shown that 90 percent of the seed crop is being produced by only 20 percent of the clones (10 top producing clones). On the basis of total tree diameter--934 inches--treatment of the top 10 producing clones would require only 467 pounds of Furadan®. The savings would be 86.7 percent over treatment of the entire seed orchard.

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[3]Experimental area established by Dr. Jack T. May, School of Forest Resources, Univ. of Ga., Athens, Ga.

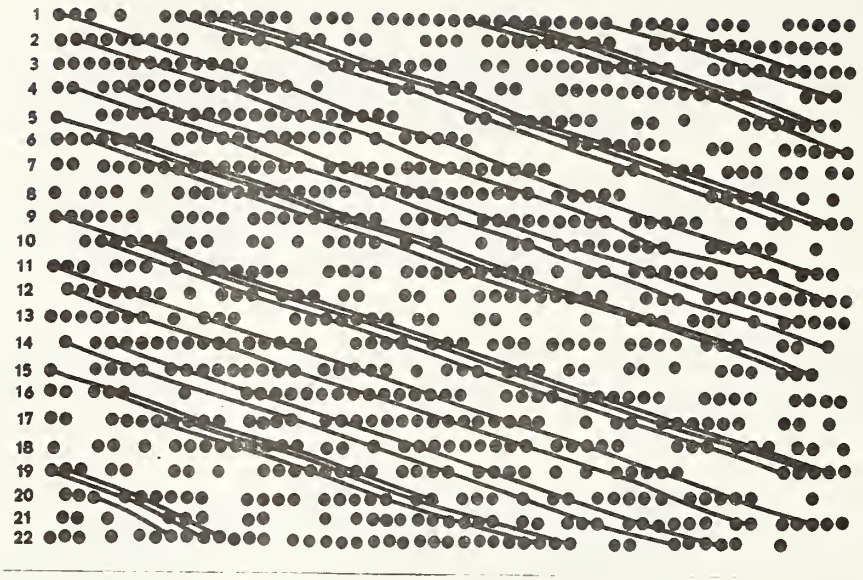

Figure 8.--Circles represent living trees in a 12-acre research block of shortleaf pine at Beech Creek Seed Orchard, Murphy, N. C. Row numbers are indicated at the left. Lines bisecting circles identify trees which belong to the 10 highest producing clones. Protection of these 122 trees would protect 90% of the cone crop.

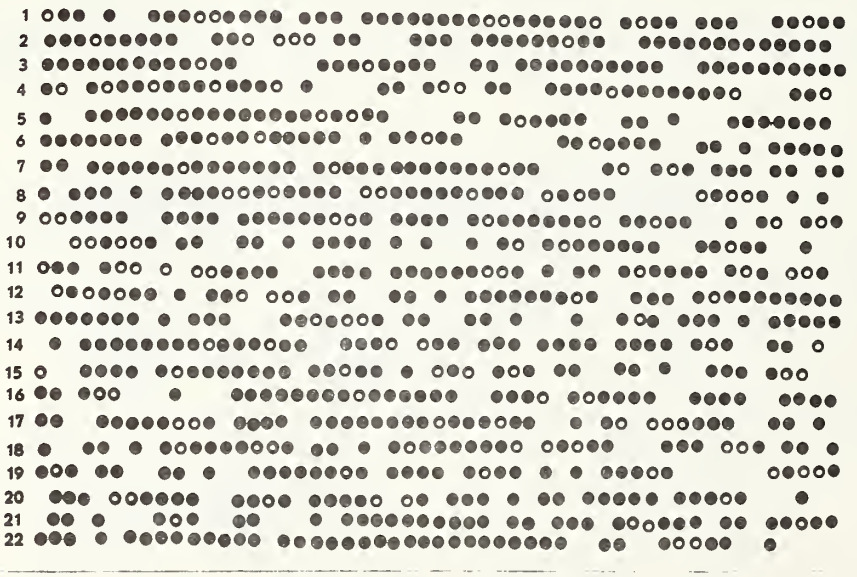

Figure 9.--White dots superimposed on dark circles indicate the location of top producing trees (145 trees) which support 90% of the cone crop.

If the "D 90/10 rule" is applied to individual trees, we find that 90 percent of the cones in the seed orchard are being produced by only 145 trees. Locations of these trees are indicated by white dots superimposed on dark circles (Fig. 9). These trees occupy only 12.4 percent of the tree locations in the study area. This is a fairly close approximation of the proposed rule.

How much of a savings of insecticide is made by treating just these 145 trees? Total diameter of these trees is 680 inches, which represents only 20 percent of the total seed orchard tree diameter. Treatment would therefore require 340 pounds of insecticide for protection (680 inches x 8 ounces). Recall that treatment of the complete seed orchard to protect 100 percent of the seed crop using a mechanized applicator required 3500 pounds of insecticide. Selecting the top producing trees, then, could achieve a 90.3 percent savings of insecticide (340 lbs. versus 3500 lbs.).

The situation may be best illustrated by Figure 10. The vertical scale is in percent and the horizontal scale is in number of trees. The vertical line to the right delimits the total seed orchard tree population of 875 trees. Tree population percentage is shown by the heavy line extending upward from "0" at a 45 degree angle. The dot-dash line represents the percent accumulated cone production. Accumulated tree diameter is indicated by a slant-dash line. With this graph any standard of cone protection can be established for this seed orchard block and the number of trees we need to protect and the quantity of insecticide required to meet this standard determined. The shaded area added to the graph delimits these quantities for protection of 90 percent of the cone crop as proposed in the "D 90/10 rule."

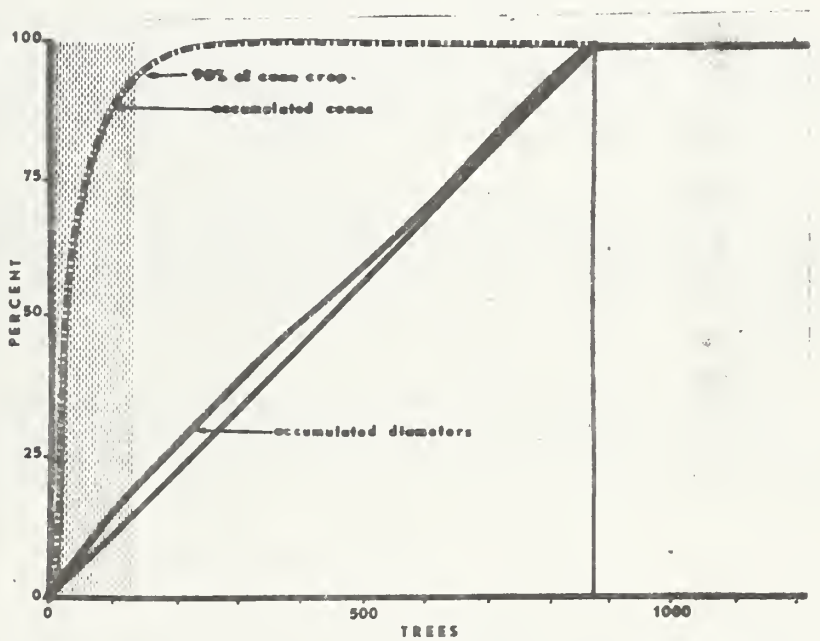

Figure 10.--Cone production (dot-dash line) and tree diameter (slant-dash line) profiles for research block at Beech Creek Seed Orchard, Murphy, N. C. Shaded area delimits number of trees and their total tree diameter which needs treatment to protect 90% of the total cone crop.

I do not mean to suggest either that the results in this particular seed orchard are necessarily typical or that the "D 90/10 rule" should in all cases be closely followed in seed orchards. Trees at the Beech Creek Seed Orchard are quite young, and production might be more evenly distributed among trees that are more mature. Furthermore, some clones may have characteristics that justify protection of small numbers of cones. What I am saying is that the orchard manager should consider how many cones he is protecting before he applies large amounts of insecticide.

For years we have argued, and rightly so, that a seed orchard is not a forest but a unique high value area that lends itself to the application of pest control techniques which are economically impractical in the forest. It is this rationalization that has encouraged the development and use of pesticides as the backbone of our seed orchard pest control programs. Certainly these chemicals will continue to figure prominently in the future, largely because pesticides presently provide the only acceptable solution to our seed orchard pest problems.

However, in view of an increasing national concern about the effects of pesticides on the ecosystem and human health, we need to be more judicious in the use of pesticides.

In summary, I would like to underscore the following points.

1. Seed orchard managers must have some form of product inventory and accountability. Life tables can meet this requirement. These will provide information on initial crop size, identity of pests causing losses, when these losses occur, and their magnitude. Until such information is known, credible pest management decisions are not possible.

2. Decisions to apply or continue pest control actions must be based upon crop size, expected pest-caused losses, and an assessment of the effectiveness of control procedures. Establishment and maintenance of check or "Judas plots" within the seed orchard is the only likely way this baseline information can be obtained.

3. Selective product protection, either on a clone or individual tree basis, should be considered. This system of deciding which trees to treat will give the best return for the money and have the least unfavorable environmental effects.

LITERATURE CITED

Bramlett, D. L., E. W. Belcher, Jr., G. L. DeBarr, G. D. Hertel, R. Karrfalt, C. W. Lantz, T. Miller, K. D. Ware, and H. O. Yates, III. 1977. Cone analysis of southern pines--a guidebook. USDA Gen. Tech. Rep. SE-__. Southeast. For. Exp. Stn., Asheville, N. C., and Southeast. Area, State & Private Forestry, Atlanta, Ga. (In Press).

DeBarr, G. L. 1971. The value of insect control in seed orchards: some economic and biological considerations. Eleventh South. For. Tree Improv. Conf. Proc. 1971: 178-185.

DeBarr, G. L., and L. R. Barber. 1975. Mortality factors reducing the 1967-1969 slash pine seed crop in Baker County, Florida--a life table approach. USDA For. Serv. Res. Pap. SE-131, 16 p. Southeast. For. Exp. Stn., Asheville, N. C.

Dinus, R. J., B. A. Thielges, and O. O. Wells. 1973. Southern forest tree improvement problems and research priorities. Twelfth South. For. Tree Improv. Conf. Proc. 1973: 348-352.

Ebel, B. H., T. H. Flavell, L. E. Drake, H. O. Yates, III, and G. L. DeBarr. 1975. Seed and cone insects of southern pines. USDA For. Serv. Gen. Tech. Rep. SE-8, 40 p. Southeast. For. Exp. Stn., Asheville, N. C., and Southeast. Area, State & Private Forestry, Atlanta, Ga.

Ebel, B. H., and H. O. Yates, III. 1974. Insect-caused damage and mortality to conelets, cones, and seed of shortleaf pine. J. Econ. Entomol. 67: 222-226.

Lakein, A. 1973. How to get control of your time and your life. Signet. The New American Library, Inc., N. Y., N. Y. 160 p.

Metcalf, R. L., and W. H. Luckmann. 1975. Introduction to insect pest management. John Wiley and Sons, New York. 587 p.

Rabb, R. L. 1972. Principles and concepts of pest management. pp. 6-29. In implementing practical pest management strategies. Proc. Nat'l Extension Pest-Management Workshop. Purdue Univ., Lafayette, Indiana.

Yates III, H. O., and B. H. Ebel. 1977. Insect-caused damage and mortality to conelets, cones, and seed of loblolly pine. (To be submitted to J. Econ. Entomol.)

DIORYCTRIA DAMAGE IN SLASH PINE SEED ORCHARDS

Suzanne E. Goldman[1/]

Abstract.--Cones from 4 International Paper Company (IPCo) slash pine seed orchards were examined for Dioryctria spp. damage as they were processed at the seed extractory. Dioryctria damaged cones ranged from 22% to 49% among the 4 orchards. Comparisons indicate that orchards established in agricultural areas had significantly less Dioryctria damage than orchards established amid cone-producing pine stands.

Additional keywords: Pinus elliottii Engelm., cone insects, natural control, insect populations, Guthion® spray program.

Cones of slash pine, Pinus elliottii Engelm., are frequently attacked or destroyed by insects. The most common cone-infesting insects are larvae of Dioryctria spp. To assess the amount of insect damage, the mature cone crop can be examined at harvest (Merkel and DeBarr 1971; DeBarr, et al 1972; and DeBarr 1974). The figures obtained, however, can only be considered as relative estimates since previous studies have shown that harvest tallies underestimate the total impact of Dioryctria on second-year cone crops (DeBarr 1974). This study was designed to estimate the amount of Dioryctria damage to cones harvested in 1976 from 4 IPCo slash pine seed orchards.

METHODS

Cones were analyzed from 4 slash pine seed orchards in 2 geographic areas. Two orchards are located in southern Alabama: Gateswood Seed Orchard (6.9 ha) in Baldwin Co., and Jack Springs Seed Orchard (6.1 ha) in Escambia Co. Two others, Dellwood Seed Orchard (12.1 ha) and Bellamy Seed Orchard (10.5 ha) are located in Jackson Co., Florida. The Jack Springs and Bellamy orchards were established in the middle of agricultural fields. The Gateswood and Dellwood orchards, however, were established amid cone-producing pine stands. Gateswood, Dellwood, and Jack Springs contain trees averaging 14-16 years old, and trees at Bellamy average approximately 8 years old.

Initially, a 100% cone tally was made as cones were processed at the Southlands Experiment Forest's seed extractory. Based on the distribution from the 100% tally, a sample size was chosen so that the proportion of damaged cones could be determined (±5%) at a 95% confidence level. Cones were randomly selected from every bushel in each lot. The number of sample cones per bushel was dependent on the total number of bushels in each lot. In each orchard,

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[1/] Forest Entomologist, International Paper Company, Southlands Experiment Forest, Bainbridge, Georgia.

cones from clones resistant to fusiform rust, Cronartium fusiforme Hedgc. and Hunt ex Cumm., were collected separately from fusiform rust susceptible clones. These two lots were analyzed separately.

Each sample cone was placed in one of the following damage classes according to the amount of Dioryctria damage: Class 0 - no damage; Class 1 - one Dioryctria boring hole; Class 2 - two Dioryctria boring holes; Class 3 - three or four Dioryctria boring holes; and Class 4 - a totally damaged cone. The percent of damaged cones by class was then determined for each orchard. Comparisons were made among orchards for damage classes 1 through 4 and the total amount of Dioryctria damage per orchard.

RESULTS AND DISCUSSION

Dioryctria damaged from 22% to 49% of the 1976 slash pine cone crop (Table 1). No significant differences were found in Dioryctria damage between fusiform rust resistant and fusiform rust susceptible lots; therefore, they were grouped together by orchard for final analysis. All orchards had more damaged cones in Class 1 than in the other three classes. Damage Class 4 exceeded Classes 2 and 3, in all orchards. This was expected since Class 4 cones often sustained additional damage from factors other than Dioryctria. Sartor and Neel (1971) reported that seed yields from Dioryctria-infested cones are almost nil and are considered a total loss. Observations in this study indicated that Class 1 cones partially open and yield some seed, whereas Classes 2,3, and 4, seldom, if ever, open. The percentage of cones in Classes 2, 3, and 4, therefore, reflect complete seed loss. The total amount of insect damage differed among orchards. Jack Springs and Bellamy had significantly lower infestation levels than Gateswood and Dellwood.

In the early 1970's with the initiation of a Guthion® spray program in both Gateswood and Dellwood seed orchards, a reduction in the amount of Dioryctria damage was observed (Merkel, et al 1976). In an attempt to explain the current high levels of Dioryctria damage and the differences among orchards, each orchard's cone harvests and insecticide spray programs for 1975 and 1976 were examined. In 1976 Gateswood produced approximately 47.9 hl of cones per ha and Jack Springs produced approximately 30.3 hl of cones per ha. Cone production levels in these 2 orchards did not differ significantly during 1975 and 1976. Both orchards were sprayed, by the same crew, with a mist blower 6 times each year (April, May, June, July, August, and October). A 1% Guthion® solution was applied to opposite sides of each ramet at the rate of 3.8 l of spray per ramet. The difference in insect control between these 2 orchards most likely reflects variation in the abundance of insects in each area. Jack Springs was established in an area surrounded by fields while Gateswood was established within a 50-year-old longleaf pine stand.

Dellwood and Bellamy were sprayed, by a second crew, with a mist blower 6 times each year. A 1% Guthion® solution was applied in the same months and at the same rates as above. Dellwood's slash pine crop was lower in 1976 than in 1975 (35.94 hl of cones per ha and 43.34 hl of cones per ha respectively). Generally, insect damage is believed more abundant when a small cone crop follows

Table 1. Dioryctria spp. damage to the 1976 slash pine cone crop in 4 IPCo seed orchards.

Orchard	Damage Classes 1	2	3	4	Total Damage
Dellwood	19.9[1/] [2/]	9.8	7.8	11.1	48.5
Gateswood	19.4	9.6	5.5	9.7	44.2
Jack Springs	14.0	5.1	3.5	7.9	30.5
Bellamy	14.8	1.2	0.0	5.8	21.9

[1/] Percents

[2/] Numbers joined by bars are not significantly different at the 95% confidence level.

a bumper crop. Dellwood's drop in production by itself would not account for the high percentage of insect damage. Bellamy's cone crop has been increasing since 1971 with 27.1 hl of cones produced in 1976. Established in an old agricultural area, Bellamy is still surrounded primarily by agricultural fields and a few mixed hardwood stands. The closest pine stands, located approximately 91 m away from one corner of the orchard, are not yet producing cones. Thus, when Bellamy came into production, a large Dioryctria population probably was not present in the area. Additionally, in Bellamy, a delayed-density dependent relationship is probably operating (DeBarr and Barber 1975). A smaller infestation rate would, therefore, be expected until the cone production levels off and the insect population catches up. Dellwood, until 1976, was surrounded by mature pine stands. A 91 to 122 m buffer area was cleared around the orchard between 1973 and 1976. The adjacent pine stands still would have influenced Dioryctria levels in the orchard, however. Once again insect abundance appears to be partially related to the areas surrounding the orchards.

SUMMARY AND CONCLUSIONS

Dioryctria damage levels were quite high in the 4 seed orchard cone crops examined. These damage levels suggest: (1) Guthion® was ineffective in preventing Dioryctria damage, and the need exists to investigate the application and timing of the individual orchard spray programs; (2) the possibility of Dioryctria resistance to Guthion® in the orchards as a result of these multiple, annual sprays; and (3) orchards established in agricultural areas have less Dioryctria damage than those established in pine stands. Studies are needed to assess the relative seasonal abundance and migration behavior of insects in order to select low risk sites for the location of future seed orchard complexes. However, these preliminary observations suggest that seed orchards established in agricultural areas may remain relatively free from Dioryctria damage and that cone bearing pine stands near orchard sites should be removed as early as possible to minimize insect abundance by the time the orchards reach cone bearing age.

LITERATURE CITED

DeBarr, G. L. 1974. Harvest counts underestimate the impact of Dioryctria on second year slash pine cone crops. USDA For. Serv. Res. Note SE-203. 3 pp.

DeBarr, G. L. and Barber, L. R. 1975. Mortality factors reducing the 1967-69 slash pine seed crop in Baker County, Florida--a life table approach. USDA For. Serv. Res. Pap. SE-131. 16 pp.

DeBarr, G. L., Merkel, E. P., O'Gwynn, C. H., Zoerb, M. H., Jr. 1972. Differences in insect infestation in slash pine seed orchards due to phorate treatment and clonal variation. For. Sci. 18:56-64.

Merkel, E. P., and DeBarr, G. L. 1971. Trunk implantations of dictrophos for cone-insect control in slash pine seed production stands. J. Econ. Entomol. 64:1295-8.

Merkel, E. P., DeBarr, G. L., and O'Gwynn, C. H. 1976. Mist blower application of Guthion® for cone insect control in slash pine seed orchards. USDA For. Serv. Res. Paper SE-148. 8 pp.

Sartor, C. F., and Neel, W. W. 1971. Impact of Dioryctria amatella on seed yields of maturing slash and loblolly pine cones in Mississippi seed orchards. J. Econ. Entomol. 64:28-30.

A NEW CONE MIDGE, CECIDOMYIA SPP. (DIPTERA: CECIDOMYIIDAE) AFFECTING SLASH PINE CONES

Isadore L. Williams and Carl W. Fatzinger[1/]

Abstract.--In 1976, first-year slash pine (Pinus elliottii Engelm. var. elliottii) cones in a northeast Florida seed orchard developed hypertrophied scales. The same symptoms appeared again in 1977. Dissection of the distorted cones revealed an infestation by cone midge larvae. The 1976 damage was found on both trees treated with carbofuran (Furadan®) and on untreated trees. In 1977, damage was observed on the same trees as well as Orthene®-treated trees. Impact data is being obtained to evaluate the necessity for control measures.

Additional keywords: Cecidomyiidae, Cecidomyia, midge, slash pine cone, seed orchard.

Very few midges have been identified as pests of pines in the South. Although adults of the gall midge family (Cecidomyiidae) partially damage or destroy first- and second-year cones of slash pine (Pinus elliottii Engelm. var. elliottii), they have not been significantly abundant in slash pine seed orchards to warrant intensive research (Ebel, 1963; Ebel et al., 1975). In 1976, hypertrophied cones appeared on trees in a slash pine seed orchard of Container Corporation of America near Callahan, Nassau County, Florida. The cone scales were grossly enlarged and protruded far beyond the normal surface of the cone.

METHODS

The first damage was observed[2/] on trees being sampled to evaluate the effectiveness of the carbamate insecticide, carbofuran (Furadan®). In 1976, the treatments were applied to four ramets of each of six of the largest clones in the orchard. Two ramets were treated with Furadan® and two ramets were left untreated as checks. Furadan® 10G was disced into the soil under the crown drip line at a rate of 178.6 g. A.I./cm. d.b.h. (1 lb. A.I./in. d.b.h.) during the first week of March 1976 and during December 1976. Hypertrophied cones were found on the Furadan®-treated trees and on the check trees. In 1977, two additional ramets of each of the same clones were treated with the systemic insecticide Orthene®. Orthene® (75 percent water soluble powder) was sprayed at the rate of 1 Kg. A.I./210 l. water (4 lb. A.I./100 gal. water) on the crowns of these trees until their foliage was dripping wet.

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2/ The damage was observed by Robert Mantie, Research Forester for Container Corporation of America, Callahan, Nassau County, Florida, while making monthly observations on trees in a carbofuran study.

The treatment was applied during the first week of January 1977 and the last week of February 1977. These trees were located about 73 m. (80 yards) from the carbofuran treatment. Like the previous year, both treated and check trees showed damage symptoms in 1977.

Hypertrophied first- and second-year cones were collected in 1976 and 1977 for determination of the cause of abnormality. Cones collected were either dissected, held in containers for future reference to observe emergence of adult insects, or preserved in 80 percent ethanol. All insect specimens dissected from the cones (larvae) and those collected from the emergence containers (adults) were sent to Dr. Raymond Gagné, United States Natural Museum, Beltsville, Maryland for identification. Damaged conelets and cones are currently being collected monthly to secure any other insects and to determine the number of generations per year.

RESULTS

Dissection of the cones revealed orange larvae about 3 mm in length and small feeding cavities below the surface of the scale. Larvae were observed feeding on the seed wing tissue and on the surface of the scales in the interior of the cone, but not on the ovules. As many as six larvae were found feeding in one cavity, and each scale above a feeding cavity was enlarged and protruded far beyond the normal surface of the cone.

The larvae were tentatively identified by Dr. Gagné as Cecidomyia spp., possibly resincola (O. S.). He currently believes them to be a new species which he plans to describe.

The infestation was first noted in May 1976 and new attacks continued to be observed on the first-year conelets until July 1976. During 1977, attacks occurred on the first-year conelets from March through June. A total of 200 conelets or 31.5 percent of the 634 sample conelets were attacked on the Furadan®-treated trees in 1976 and 25.5 percent of these conelets died (Table 1). In comparison, only 34 (6.1 percent) of the 557 sample conelets on the check trees were attacked in 1976 of which 23.5 percent died. During 1977, 18.2 percent of the 1222 sample conelets on Furadan®-treated trees were attacked of which 19.7 percent died, 8.5 percent of the 579 sample conelets on Orthene®-treated trees were attacked of which 40.8 percent died, and 5.8 percent of the 825 sample conelets on the check trees were attacked of which 18.8 percent died.

The insect does not appear to favor any side of the tree (cardinal direction)3/, and the damage usually cannot be seen from the ground. Some cones have only one enlarged scale and can easily be overlooked.

3/ Personal communication with Robert Mantie.

Table 1.--Numbers of first-year conelets sampled that were attacked by midges and numbers subsequently killed during 1976 and 1977 in a slash pine seed orchard in northeast Florida.

Month	1976				1977					
	Furadan®		Check		Furadan®		Orthene®		Check	
	No. attacked	No. killed	No. attacked	No. killed	No. attacked	No. killed	No. attacked	No. killed	No. attacked	No. killed
Mar.							38			
Apr.					119	15		5	15	3
May	174		23		76	24	5	12	19	6
June	22	6	8		28	5	6	3	14	
July	4	4	2	2						
Aug.		10		1						
Sept.		12		2						
Oct.		3		1						
Nov.			1							
Dec.										
Jan.										
Feb.										
Mar.		7								
Apr.		9		2						
Total	200	51	34	8	223	44	49	20	48	9

DISCUSSION

Feeding by larvae of the cone midge appeared to stimulate hypertrophy of cone scale tissues. Larvae were found in all of the hypertrophied cones dissected. The larvae do not appear to feed directly on seeds, but their feeding activity does cause cone mortality, thus reducing seed orchard yields.

Although the cone midge infestation appears to be severe in this orchard, it is not known to be widespread geographically. We are presently evaluating the impact of the cone midge on seed yields and seed quality to determine whether control methods will be required in the future.

LITERATURE CITED

Ebel, B. H.
1963. Insects affecting seed production of slash and longleaf pines--their identification and biological annotation. Southeast. For. Exp. Sta., USDA For. Serv. Res. Pap. SE-6, 24 pp.

Ebel, Bernard H., Raymond J. Gagné, and Edward P. Merkel.
1975. Cecidomyiidae from pine cones in Florida, with a generic key to larvae. Fla. Entomol. 58(3):193-197.

This publication reports research involving pesticides. It does not contain recommendations for their use nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

EFFECT OF AN INSECTICIDE SPRAY PROGRAM ON SLASH PINE SEED ORCHARD EFFICIENCY

John F. Godbee, Jr.,[1/] Terry S. Price,[1/] and David L. Bramlett[2/]

Abstract.--The efficiency of slash pine production was compared in Guthion®[3/] sprayed vs. unsprayed plots in the Georgia Forestry Commission's Horseshoe Seed Orchard. Guthion was applied monthly from April to September for 1975 and 1976. The primary losses were from pre-harvest mortality of flowers, conelets, and from the abortion of ovules before seed maturity. Guthion increased cone and seed efficiencies to 58 and 39 percent, respectively, compared to values of 38 and 24 for the unsprayed plots. Extraction and germination efficiencies were 90 and 83 percent for the Guthion plots compared to 88 and 78 for the unsprayed plots. Overall production was evaluated by combining separate efficiencies for cone survival, filled seed/cone, seed extraction, and seed germination. On this basis, the seed orchard to nursery efficiency was 17 percent in Guthion spray plots, and only 6 percent for the unsprayed plots.

KEYWORDS: Azinphosmethyl, cones,conelets, ovules, insects.

Seeds from southern pine seed orchards are the vital link between selected parents and a newly established pine plantation of improved genetic capability. Consequently, the seed has high economic value as the sources of increased growth, wood quality and pest resistance. This high value and importance of the seed crop justifies considerable effort to protect the seed crop in the orchards from flower initiation through seed germination.

In 1965, Merkel and Yandle first reported that Guthion applied by a mist blower effectively controlled Dioryctria spp. (Lepidoptera: Pyralidae: Phycitinae) and the slash pine seed worm, Laspeyresia anaranjada Miller (Lepidoptera: Olethreutidae), in cones of slash pine, Pinus elliottii Engelm. Subsequent tests on longleaf pine P. palustris Mill, were conducted by DeBarr and Merkel (1971). Additional data on Dioryctria spp. control and on the effectiveness of Guthion in protecting maturing seed within the cone from losses caused by the seedbugs Leptoglossus corculus (Say) (Hemiptera: Coreidae) and Tetyra bipunctata (Hemiptera: Pentatomidae) was obtained by Merkel and others 1976. This study evaluated an operational test of Guthion in increasing seed orchard efficiency.

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3/ Guthion® - registered trade name for azinphosmethyl.

METHODS AND MATERIALS

The Horseshoe Bend Slash Pine Seed Orchard, Wheeler County, Georgia was divided into four blocks, each containing four plots (seed orchard block). The orchard is approximately 35 acres in size with trees averaging 18 years of age. Treatments were randomly selected with one control and three sprayed plots located in each block. A mist blower was used to apply Guthion 2L as a .24 percent water emulsion with the addition of 8 ounces per 100 gallons of Security spreader-sticker. The mist blower was calibrated to deliver 58 gallons per acre. All sprayed treatments received operational orchard insecticide spray at approximately monthly intervals (varied from 22-43 days depending on weather, equipment, etc.) from April-September of each year.

Two ramets each of three clones were chosen at random from each plot for a total of 96 sample trees. Initial counts of female strobili (flowers) produced on each sample tree were made in February, 1975. A subsample of approximately 25 percent of the total flowers produced was selected by systematically tagging 10 sample branches distributed throughout the flower production area. Periodic sample counts were made in February, April, May, July, and September, 1975 and February, May, July, and September, 1976. The causes of strobili mortality were recorded if insect damage was evident (DeBarr and Barber 1975). If no evidence of insect damage was distinguishable, mortality was classified as unknown.

All mature cones were collected in September, 1976. Three healthy cones were chosen at random from each tree. The selected cones were analyzed with the cone analysis procedure (Bramlett 1974, Bramlett and others 1977) to determine the seed potential, aborted ovules, developed seed, filled seed, and types of seed losses. Germination tests were conducted on all seed for 30 days. Analyses of variance were used to evaluate the effect of the Guthion treatment on the cone and seed variables.

RESULTS

Cone Efficiency and Mortality

The survival from flowers to cones was directly related to the Guthion treatment (table 1). The overall yield of sound cones indicated an increased survival from flowers to cones in treated trees. An average of 71 sound cones was produced per tree in Guthion treated plots compared to 60 per tree in the control. Cone efficiency (CE), the ratio of harvested healthy mature cones to pollinated flowers increased from 38 percent in the control to 59 percent when test trees were sprayed with Guthion. The effect of the Guthion treatment on cone efficiency was significant at the 0.05 percent level.

Table 1.--Survival of slash pine conelets and cones on sample branches in Guthion versus control treatments over a two-year period

Sample date	Cone survival: Guthion[1/]	Cone survival: Control[2/]	Analysis of variance
	----------Percent----------		
2-75	100	100	ns
4-75	93	91	ns
5-75	92	89	ns
7-75	91	85	ns
9-75	86	79	ns
2-76	78	64	*
5-76	72	48	**
7-76	72	44	*
9-76	59	38	*

1/ 72 trees in sample averaging 33 flowers tagged.
2/ 24 trees in sample averaging 32 flowers tagged.

* Treatment differences in an analysis of variance were significant at the 0.05 level. Degrees of freedom to test treatment effects were one for treatments and twelve for error term plot (block x treatment) .
** Significant at the 0.01 level.
ns Non-significant.

First year losses of 21 percent of the total flower crop in the control and 14 percent in the Guthion treated plots were recorded from flower to conelets. Thrips destroyed 1 percent of the total flowers in both control and Guthion treatments. This loss occurred from February to April or before insecticide sprays were initiated. Dioryctria spp. damaged 1 percent of the total flowers in both control and Guthion treatments. Losses attributed to the pine conelet looper, Nepytia semiclusaria (Walker), May beetles, Phyllophaga spp., pitch canker, Fusarium spp., and mechanical accounted for 5 and 3 percent in the control and Guthion treatment respectively. Seven percent of the control and 4 percent of the Guthion treated conelets were dead without visible insect damage. Seven percent of the control and 5 percent of the Guthion treated conelets were recorded as missing. Cone efficiency of the first year conelets was 79 percent in the control and 86 percent in the Guthion treatments. This difference was non-significant in an analysis of variance.

Second year cone losses were 41 percent of the original total flower crop in the control and 27 percent in the Guthion treated plots. Initial counts in February, 1976 showed cone efficiencies of 64 and 79 for control and Guthion treatments respectively. Late fall and overwintering losses combined with first year mortality were significant at the 0.05 percent level.

Dioryctria spp. damage amounted to approximately 6 percent of the second year cones lost in the controls and 5 percent in the Guthion treatment. Seven percent of the damaged cones in the control and 4 percent in the Guthion treated plots showed no evidence of insect damage. Missing cones accounted for the largest category of losses with 28 percent of the control and 18 percent of the Guthion treated cones in this category.

Seed Efficiency

Seed efficiency (SE) measured the seed production of a cone in relation to the biological capacity. This value was expressed as the ratio of filled seed to potential seed and is the single most important value when measuring seed production.

Average seed production per cone in the control increased from 64 out of a seed potential of 176 to 92 of a possible 166 when Guthion was used as a spray. The average number of filled seed per cone increased from 43 in the control to 66 in the Guthion treatment (table 2). This difference was significant at the 0.01 percent level and reflects an increase from 24 to 39 percent in seed efficiency.

Table 2.--Mean number of seed and ovules per cone as classified in cone analysis in control versus Guthion treated plots

Seed and ovule classification	Average per cone		Analysis of variance
	Guthion 1/	Control 2/	
Aborted 1	64	91	**
Aborted 2	11	21	**
Total seed	92	64	**
Filled seed	66	43	**
Empty	18	15	ns
Seed bug	2	1	ns
Fungus	3	3	ns
Laspeyresia	1	1	ns
Abnormal	3	1	ns

1/ 216 cones in sample.
2/ 72 cones in sample.

** Significant at the 0.01 level. Degrees of freedom to test treatment effects were one for treatments and twelve for error term, plot (block x treatment) .

ns Non-significant.

Major loss categories were identified as aborted ovules or developed seed that were not classified as filled. First year aborted ovules accounted for the majority of seed losses in both treatments. An average of 91 first year aborted ovules per cone was found in controls as compared to 64 per cone in the Guthion treatment. The average number of second year aborted ovules decreased from 21 for the control to 11 when Guthion was applied. The effect of the Guthion treatment on total seed, seed efficiency, first and second year aborted ovules, was significant at the 0.01 percent level. Differences in other losses were attributed to empty seed, seedbug, fungus[4/], Laspeyresia spp. and abnormal seed and were non-significant when controls were compared to Guthion treatment (table 2).

Extraction Efficiency

Extraction efficiency (EE) was expressed as the ratio of developed seed which are removed by the drying and extracting procedure to the total seed in the cones. The cones evaluated in this study opened well with an average of 89 percent of the seed per cone extracted. Averages of 88 and 90 percent were found when comparing cones from control to those from treated blocks. Analysis of variance showed no significant difference between treatments.

Germination Efficiency

The germination efficiency (GE) evaluated the viability of filled seed. It is expressed as a ratio of the number of germinated seed to the number of filled seed produced by a cone. Germination increased from 78 percent in the control to 83 percent in the sprayed plots. This difference was non-significant in an analysis of variance.

DISCUSSION

Seed Orchard to Nursery Efficiency

The seed orchard to nursery efficiency (SO-NE) was used to evaluate the cone, seed, and seedling efficiency of the sprayed and unsprayed plots in the Horseshoe Seed Orchard. The SO-NE was calculated as a product of the four separate efficiency values as follows:

$$SO\text{-}NE = CE \times SE \times EE \times GE$$

This evaluation procedure allowed the orchard manager to monitor the overall seed orchard performance by comparing the cumulative efficiency to the biological reproductive potential for the orchard. On this basis, the SO-NE for the sprayed plots was 17 percent compared to only 6 percent for the control plots (fig. 1).

4/ Seed with evidence of fungal mycelium identified as Diplodia spp. and Fusarium spp. by Tom Miller, USFS, SEFES, Athens, Georgia, Personal Communication.

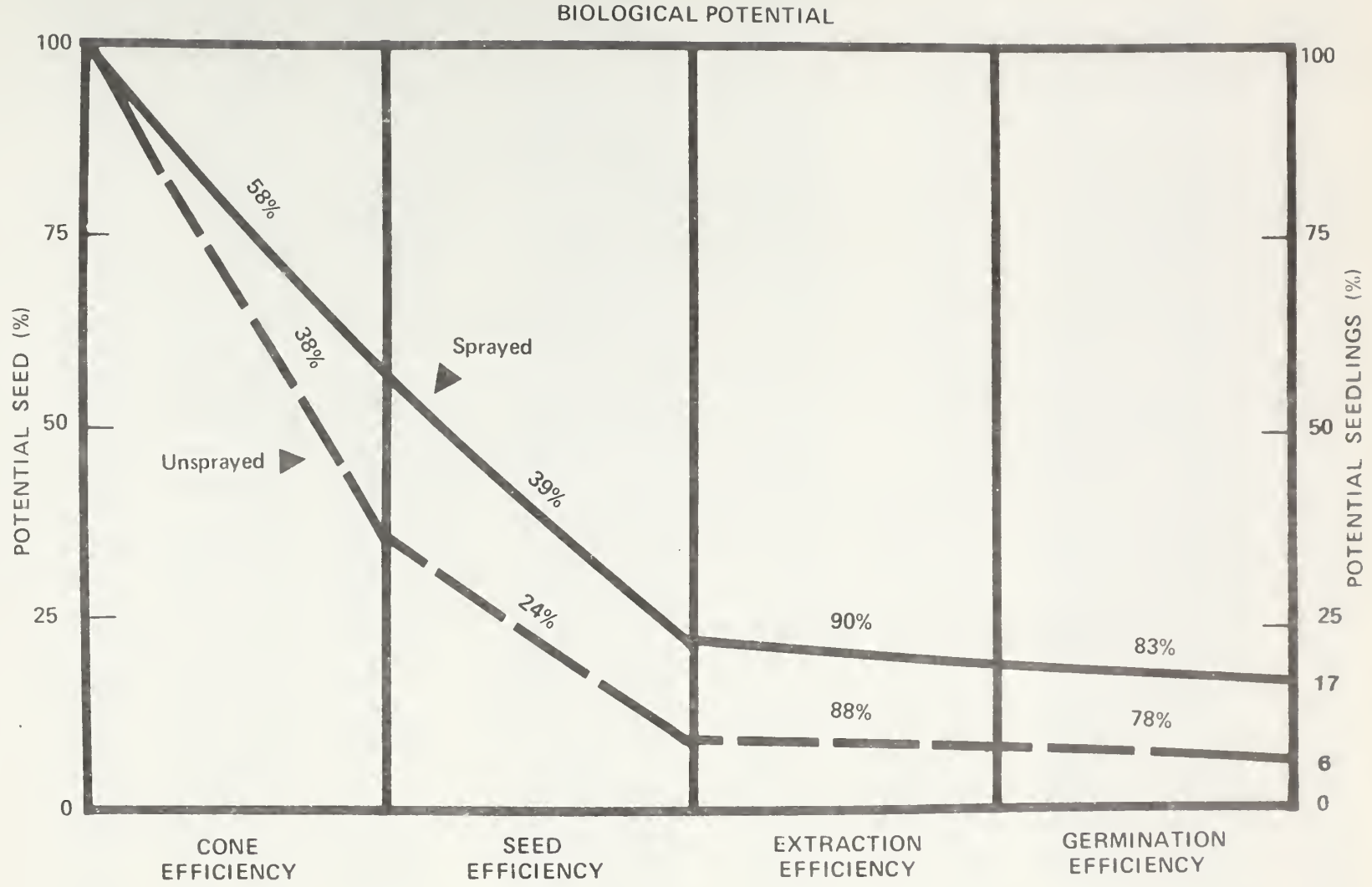

Figure 1.--Seed orchard to nursery efficiency of slash pine cones in Guthion® vs. control treatments.

In this study, the two primary stages of seed losses were identified. First the loss of flowers, conelets, and cones before maturity greatly reduced the cone efficiency. From the observed dates and insect damage, the losses were primarily from the apparent seedbug damage of conelets that were missing or aborted during late summer and fall. Secondly, the high number of aborted ovules in the mature cones reduced the seed efficiency to relatively low values. The insecticide spray program significantly reduced the losses but cones from both areas had a low seed efficiency.

Both the extraction efficiency and germination efficiency were relatively high and were not related to the insecticide spray program. Unfortuantely, the high EE and GE had only limited benefit in the seed orchard because the cone and ovule losses that occurred early in the seed development cycle had already reduced seed yields to low levels.

Certainly the seed production process cannot be expected to reach 100 percent efficient. Yet, it is obvious that seed orchards can produce at much higher efficiency values than were recorded in the study. Although the upper biological limits have not been set for seed orchard to nursery efficiency, values of 45 to 50 percent may be possible for operational seed orchards.

Seed orchard to nursery efficiency can be used in evaluation of seed orchard performance. By using the seed orchard to nursery efficiency, the orchard manager can identify the critical times and probable causes of seed losses. This information can then be directed toward management practices that reduce the losses. Seed orchard to nursery efficiency can also be used to evaluate the cost of seed orchard management in terms of increased production of seed or seedlings.

LITERATURE CITED

Bramlett, David L. 1974. Seed potential and seed efficiency, p. 1-7, In John Kraus (Ed.), Seed yield from southern pine seed orchards, Colloquium Proceedings. Ga. For. Res. Council, Macon, Ga.

Bramlett, D. L., E. W. Belcher, Jr., G. L. DeBarr, G. D. Hertel, R. Karrfalt, C. W. Lantz, T. Miller, K. D. Ware, and H. O. Yates, III. 1977. Cone analysis of southern pines--A guidebook. USDA, For. Serv. Gen. Tech. Rep. SE-____. Southeast. For. Exp. Stn., Asheville, N. C. and Southeast. Area, State and Private Forestry, Atlanta, Ga. (In Press).

DeBarr, G. L., and L. R. Barber. 1975. Mortality factors reducing the 1967-1969 slash pine seed crop in Baker County, Florida--A life table approach. USDA, For. Serv. Res. Pap. SE-131, 16 p. Southeast. For. Exp. Stn., Asheville, N. C.

DeBarr, G. L., and E. P. Merkel. 1971. Mist blower spraying of longleaf pine for cone and seed insect control. USDA, For. Serv. Res. Pap. SE-76, 7 p. Southeast. For. Exp. Stn., Asheville, N. C.

Merkel, E. P., and D. O. Yandle. 1965. Mist blower application of insecticides for cone insect control on slash pine. U.S. For. Serv. Res. Note SE-52, 4 p. Southeast. For. Exp. Stn., Asheville, N. C.

Merkel, E. P., Gary L. DeBarr, and Claude H. O'Gwynn. 1976. Mist blower application of Guthion for cone insect control in slash pine seed orchards. USDA, For. Serv. Res. Pap. SE-148, 8 p. Southeast. For. Exp. Stn., Asheville, N. C.

EVALUATION OF CARBOFURAN FOR THE CONTROL OF THRIPS, CONEWORMS, AND SEEDBUGS IN A SLASH PINE SEED ORCHARD

Edward P. Merkel,[1] Carl W. Fatzinger,[1] and Lee Draper, Jr.[2]

Abstract.--The systemic insecticide, carbofuran (Furadan® 10G), was applied to the soil at dosage rates ranging from 9.6 to 19.2 oz. of 10% granules per in. tree dbh in a slash pine seed orchard to evaluate its effectiveness in controlling the slash pine flower thrips, Dioryctria coneworms, and seedbugs. Female flower mortality caused by thrips was reduced significantly 10 months following a total dosage of 19.2 oz./in. tree dbh of Furadan® 10G applied as a split application. In three consecutive field tests (1972-1974), significant control of coneworms on first- and second-year cones was obtained only in 1973, and seedbug damage was reduced significantly only in 1974. Possible reasons for erratic coneworm and seedbug control results are discussed.

Additional keywords: Dioryctria spp.; Leptoglossus corculus (Say); Tetyra bipunctata (H. & S.); Gnophothrips fuscus Morgan; systemic insecticide; carbofuran.

In 1976, the FMC Corporation, Middleport, N.Y., obtained E.P.A. registration of Furadan® 10 Granules[3] for control of seedbugs, (Leptoglossus corculus (Say) (Hemiptera:Coreiidae) and Tetyra bipunctata (H. & S.) (Hemiptera:Pentatomidae)), coneworms, (Dioryctria spp. (Lepidoptera:Pyralidae)), cone borers, (Eucosma spp. (Lepidoptera:Olethreutidae)), and cone beetles (Conophothorus coniperda (Schwarz) (Coleoptera:Scolytidae)) in southern pine seed orchards. Prior to registration of Furadan®, i.e., 1972-1974, cooperative field tests with Container Corporation of America were conducted to obtain efficacy data for carbofuran as a control for coneworms, seedbugs, and the slash pine flower thrips, (Gnophothrips fuscus Morgan (Thysanoptera: Phlaeothripidae)), one of the more destructive insects affecting slash pine (Pinus elliottii Engelm.) seed production (Ebel 1961) (DeBarr 1969).

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[2] Research Forester, Container Corporation of America, Callahan, Florida 32011.
[3] FMC Corporation's trade name for the granular insecticide containing 10% by weight, of the active ingredient carbofuran, chemically defined as 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate.

Field tests were conducted on a 10-acre slash pine seed orchard in Nassau County, Florida owned by Container Corporation of America. Tree spacing in the orchard is 30 by 30 feet. Soil is a Plummer fine sand and ground cover is primarily Pensacola Bahia grass. Trees were 10 years of age and averaged 10 in. dbh when the first test was installed.

1972 Test.--Paired ramets of 15 different slash pine clones were chosen for this test. One ramet of each pair was randomly selected for treatment and the other ramet served as an untreated check.

On May 4, carbofuran 10% granules were applied to the soil surface within the drip area of the tree. The grass was mowed around each tree prior to applying the granules; then the granules were covered with the grass clippings to minimize the hazard to birds. The total dosage of Furadan® 10G applied was 6 lb. per tree, or an average of 9.6 oz. per in. dbh (107 gm/cm tree dbh).

On the day of the treatment and four months after treatment, 8 clusters of first-year cones in the upper southeast quadrant of each tree were examined for signs of attack by coneworm larvae. Percentages of cones infested were transformed to arcsin $\sqrt{\%}$ and analyzed by the t-test for correlated data (table 1). No significant differences were found between treated and untreated trees at the 5-percent probability level, 4 months after treatment.

Table 1.--Percentages of first-year slash pine cones, by condition catagories, on carbofuran-treated vs. untreated trees, 4 months after insecticide application. Nassau County, Florida, 1972.

Treatments	Cone-condition catagories			
	Sound	Dioryctria	Unknown	Missing
Carbofuran 10% granules, 9.6 oz./in. dbh	80.6	2.6	2.6	14.2
Untreated	79.3	3.6	2.5	14.6

1973 Test.--The experimental design, study trees, and application method were the same as for the 1972 test. Carbofuran 10% granules were again applied to the soil surface, but the total dosage rate per tree was doubled over that of the previous year; a total of 12 lb. Furadan® 10G was applied per tree. An average of 9.6 oz. per in. dbh (107 gm/cm dbh) applied twice, once on January 19 and again after 3 months on April 24.

Eight sample conelet clusters of first- and second-year cones in the upper southeast quadrant of each tree were examined for coneworm larvae attacks.

The first observations were made one month after the first treatment on February 21, and the second observations were on the day of the second treatment, April 24. The final observations were made on August 30, four months after the second treatment (2 weeks prior to cone harvest).

On August 30, percentages of first- and second-year cones attacked by coneworm larvae were significantly lower on treated trees than on untreated trees. Treatment did not significantly affect the mean percentage of seedbug-damaged seeds per cone (table 2).

On February 26, 1974, ten months after the second 1973 carbofuran application, 100 female flowers per study tree were examined for thrips-caused damage and mortality. On treated trees, flower mortality and damage from thrips were reduced by 47 percent, a significant reduction (table 2).

Table 2.--Effects of carbofuran on various parameters from treated vs. untreated slash pines. Nassau County, Florida, 1973.

Variables Tested	Mean Percentages		Statistical Significance[b/]
	Untreated	Treated[a/]	
% cones attacked by *Dioryctria* spp./tree:			
1st year conelets	3.1	0.0	*
2nd year conelets	10.6	0.6	**
% sound cones harvested/tree	82.3	92.7	***
% full seed yield/tree	58.6	66.8	n.s.
% filled seed/cone	64.3	63.9	n.s.
% seedbug-damaged seed/cone	16.0	12.5	n.s.
% empty seed/cone	21.4	25.0	n.s.
% damaged seed (empty + seedbug)/cone	37.4	37.5	n.s.
% female flowers thrips-killed and -damaged/tree[c/]	37.3	19.9	*

[a/] Total dosage of 19.2 oz./in. tree dbh of Furadan® 10G applied (half of total dosage applied January 19 and the other half applied April 24).

[b/] Single, double, and triple asterisks indicate statistical significance between treated and untreated means at the 5-, 1-, and 0.1-percent probability levels, respectively; n.s. indicates means are not significantly different.

[c/] Evaluated on February 26, 1974.

1974 Test.--Carbofuran was applied once on April 11 to slash pine that had no prior insecticide treatment. Three dosage levels of 10% carbofuran granules were used; 0-, 8-, and 16-oz. per in. of tree dbh (0-, 89.3-, and 178.6-gm/cm dbh). Each dosage was applied to a 9-tree plot and was replicated three times for a total of 81 study trees. Carbofuran granules were spread manually in a 4-foot wide band around the crown drip-line and then disced lightly into the soil.

The second-year cones were harvested from the study trees by means of a tree-shaker and examined for coneworm infestation. Coneworm infestation of second-year cones was extremely low on all study trees and no significant difference was found between the three treatments.

Six apparently sound cones were also removed from each study tree at harvest to estimate seedbug damage. Seeds were extracted, radiographed, and classified as filled, seedbug-damaged, or empty. The percentage of seeds damaged by seedbug was significantly different between treated and untreated trees. The 16 oz. per in. dosage reduced seed damage by 81 percent; the 8 oz. per in. dosage by 79 percent (table 3). The difference in mean seedbug-damaged seed per cone between the two dosage levels was not significant.

Table 3.--Effects of carbofuran on various parameters from treated vs. untreated slash pines. Nassau County, Florida, 1974.

Variables Tested	Mean Percentages			Statistical Significance[b]
	Untreated	8 oz./in. dbh[a]	16 oz./in. dbh	
% 2nd year cones attacked by Dioryctria spp./tree	0.6	1.6	3.2	n.s.
% sound cones harvested /tree	97.3	96.8	97.6	n.s.
% full seed yield/tree	66.7	81.6	82.8	*
% filled seed/cone	68.4	84.6	86.5	*
% seedbug-damaged seed/cone	16.1	3.4	3.0	*
% empty seed/cone	14.8	11.7	10.5	n.s.
% damaged seed (empty + seedbug)/cone	30.9	15.1	13.5	*

[a] Since study trees averaged 10 in. dbh, dosages were equivalent to 5- and 10-pounds of Furadan® 10G/tree or 245- and 490-pounds/acre.

[b] Significant differences were not detected between means at the 8- and 16-oz./in. dbh dosage levels; * indicates significance between untreated vs. dosage level at the 5 percent probability level; n.s. indicates non-significant differences among all means.

DISCUSSION

In 1972, coneworm attacks on first-year cones only were evaluated for carbofuran effectiveness because the second-year cone crop was too light. The inconclusive coneworm control in this test may have been due to: (1) application of the granules to the soil surface reduced the chances of effective up-take by the tree roots, and (2) the late (May 4) application date coupled with the short time (4 months) between application and final evaluation of effectiveness. The possible carry-over effect into February 1973 on thrips control was not evaluated.

In 1973, the carbofuran was applied to the soil surface around the same trees treated the previous year, but a double application at the rate of 9.6 oz./in. dbh was made in January and April. The early timing of application, as well as the increased total dosage rate in 1973, may have accounted for the significant reduction in coneworm attacks on first- and second-year cones and the significant reduction in thrips-killed and -damaged flowers the following February 1974 (10 months after the last carbofuran application). Seedbug-damaged seed was not reduced significantly in the 1973 test.

In the 1974 test, both the 8- and 16-oz./in. dbh carbofuran application rates applied once in April and incorporated into the soil significantly reduced seedbug damage, but neither dosage rate effectively controlled coneworms on maturing cones.

Possible reasons for the erratic coneworm control results in these tests include the following observations. The levels of coneworm attacks were lower than would be expected in slash pine seed orchards during the three-year period. Coneworms typically attack from 15- to 45-percent of the second-year cones (Merkel et al., 1965), but during this study, coneworm attacks on untreated trees ranged only from 0.6- to 10.6-percent. Such low levels of attack might have obscured differences between treated and untreated trees and resulted in an apparent ineffectiveness of carbofuran to control coneworms. It is also probable that more conelets and cones might have been found to be attacked by coneworms if cone-condition observations had been made at more frequent intervals, i.e., cones that were attacked, killed, and fell from the trees between observations would not be included in the data analyses.

Finally, it should be emphasized that only 1 of the 3 tests reported here conformed to the registered method of application, i.e., incorporating the granules thoroughly into the soil and in only one of the tests was the carbofuran applied in winter and early spring, as now recommended. Nevertheless, further research is warranted to obtain more efficacy data on slash pine flower thrips control so carbofuran can be registered for use against this serious flower pest.

LITERATURE CITED

DeBarr, G. L.
1969. The damage potential of a flower thrips in slash pine seed orchards. J. For. 67:326-327.

Ebel, B. H.
1961. Thrips injure slash pine female flowers. J. For. 59:374-375.

Merkel, E. P., A. E. Squillace, and G. W. Bengtson.
1965. Evidence of inherent resistance to Dioryctria infestation in slash pine. Eighth South. Conf. For. Tree Improv. Proc. 1965:96-99.

SEED ORCHARD SEED EVALUATION TESTING (SOSET) AND CONE ANALYSIS SERVICE (CAS) AT THE EASTERN TREE SEED LABORATORY

R. P. Karrfalt [1/]

Abstract.--A basic summary of the data from the first three years of SOSET and the first two years of CAS is presented. Yields of apparently sound seed from slash and loblolly pine increased by over 200 percent from 1974 to 1976. The amount of insect damage to mature seed was very small in the 1975 and 1976 cone collections. Both of these results are attributed to more intensive management practices.

Immature cone harvesting is cited as a major problem in participating orchards, indicating that substantial financial losses occur because seed is not extracted from the cones. A two part cone harvest is a suggested solution to this problem.

Seed production capacities for loblolly seed orchards appeared to decrease along an east to west gradient. Differences among individual clones within an orchard were large enough to possibly obscure geographic patterns.

A scheme is presented for integrating SOSET and CAS in a manner to efficiently evaluate the seed production of all clones in an orchard. This scheme will be useful for both short term and long term planning.

Additional keywords. Seed production capacity, seed production efficiency, cone harvesting, orchard evaluation.

INTRODUCTION

This report summarizes results obtained from the first three years of Seed Orchard Seed Evaluation Testing (SOSET) and the first two years of Cone Analysis Service (CAS). Both are service programs offered by the Eastern Tree Seed Laboratory to assist seed orchard managers. The main points of discussion deal with improvements in seed yields, categories of seed loss, improvements to be made in management practice and future utilization of these programs.

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METHODS AND MATERIALS

Loblolly (Pinus taeda) and slash pine (P. elliottii) have been the species most frequently submitted and provide the bulk of the material analyzed. Shortleaf (P. echinata), Virginia (P. virginiana), and longleaf (P. palustris) pines have been submitted for testing. Data analysis has been restricted to means and ranges because of irregularity in orchard participation and periodic changes in which clones are submitted.

The procedures used in SOSET are essentially those used for the Seed Orchard Survey (SOS) program (Belcher and Hitt 1973). The CAS procedures and concepts are presented by Lyons (1956), Bramlett (1974), and Karrfalt and Belcher (1977). The most important differentiating characteristics between SOSET and CAS are listed in table 1.

Table 1.--Differentiating characteristics between CAS and SOSET

SOSET	CAS
Clonal bulk samples of 10 to 20 cones	10 to 20 individual cone samples per clone
Analyzes only seed extracted by drying cone	Analyzes all seed produced and aborted ovules
Yields evaluated relative to other yields	Yield evaluated relative to cone's seed production capacity
Categories of seed losses incompletely determined	Categories of seed losses fully determined

RESULTS AND DISCUSSION

SOSET

A steady improvement in seed production was observed in the annual means computed from the SOSET data. Number of seeds per cone for both loblolly and slash nearly doubled during the first three years the program has been offered (table 2). The percentage of apparently sound seed (estimated by x-ray) has also shown strong improvement. In 1974, 29 apparently sound seed per cone were being produced by loblolly and 28 by slash. In 1976, these mean values had increased to 71 and 63, respectively, which amount to increases of 245 percent and 225 percent over the 1974 levels. These production figures can be compared to seed production capacity, estimated by the cone analysis, to

arrive at values for seed production efficiency. Assuming seed production capacities of 150 seeds per cone for loblolly and 163 for slash, seed production efficiencies for loblolly would be 19 percent of capacity in 1974, increasing to 48 percent in 1976. For slash pine this efficiency would be 17 percent in 1974 and 39 percent in 1976. Comparing these results to those of third year data of the SOS (Belcher 1974), we find that the efficiency of loblolly increased from 35 percent to 48 percent and for slash it increased from 18 percent to 39 percent.

The number of insect damaged seeds decline sharply while the yield of good seeds increased. Both of these trends indicate that the efforts toward more intensive orchard management are having a very positive effect on seed production from the orchards.

Table 2.--Annual means for loblolly and slash pine analyzed by SOSET

	Loblolly			Slash		
Measurement	1974	1975	1976	1974	1975	1976
Seeds per cone	56	87	102	54	96	97
% Apparently sound seed	52	71	70	52	65	65
Sound seed per cone	29	62	71	28	62	63
Seed production efficiency	19	41	48	17	38	39
Viability of filled seed	95	95	96	90	94	90
% Insect damage in extracted seed	24	1	1	28	3	2

CAS

Overall, loblolly production averages declined from 1975 to 1976 (table 3). These averages are explainable; however, by losses from the program of some high producing orchards and the gain of some orchards with production problems. Second year ovule abortions were generally very low, averaging only 2 to 3 percent of seed production capacity. A few clones had more than 5 percent second year abortions, none exceeded 10 percent. Also, insect

damage observable in mature seeds averaged only 1 percent for loblolly. Both of these results indicate better management is reducing seed losses caused by insects.

Table 3.--Cone analysis summary for loblolly pine; 1975 & 1976

Measurement	Mean[a]		High Clone[b]		Low Clone[b]	
	1975	1976	1975	1976	1975	1976
	- - - - - - - - Percent - - - - - - - -					
1st year ovule abortion [c]	27	40	48	60	17	18
2nd year ovule abortion [c]	3	2	5	8	1	0
Visible insect damage [c]	1	1	4	7	0	0
Empty seed [c]	13	15	23	9	8	26
Seed production efficiency [c]	55	41	88	79	38	4
Other	0	1	1	4	0	0
Extraction efficiency	82	67	98	99	0	0
Full seed germination	96	95	99	100	92	87
Seedling efficiency [c]	47	31	81	76	0	0
Seed production capacity	145	149	171	204	129	113

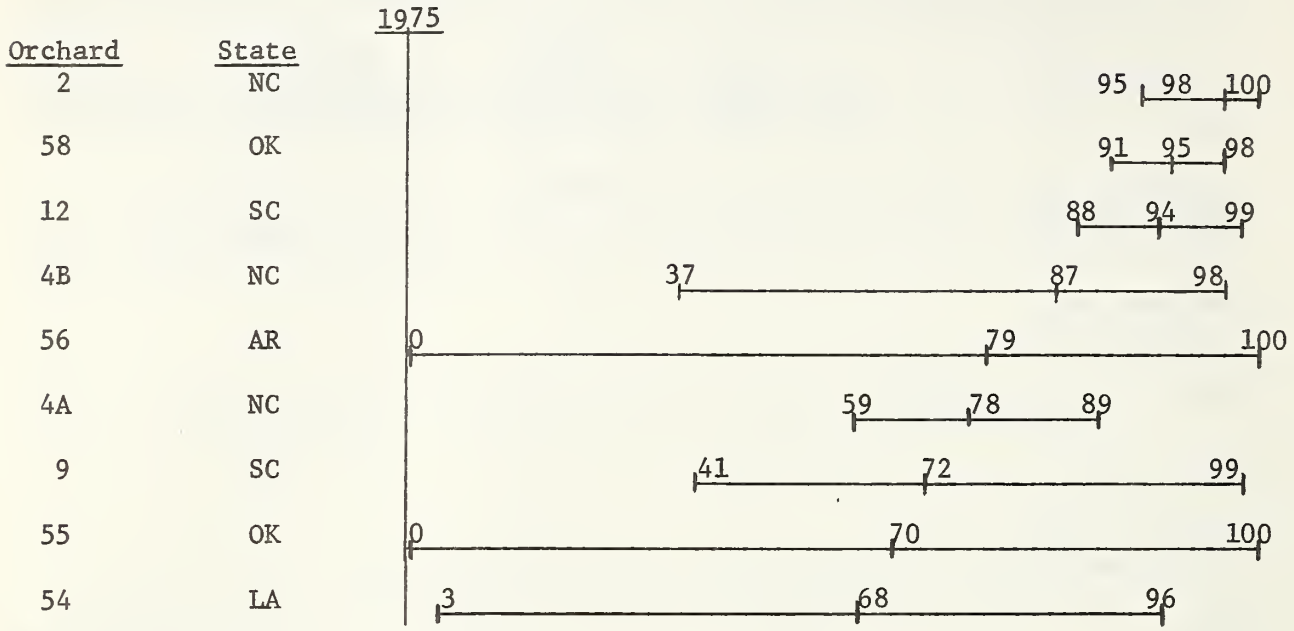
a/ Mean value for all clones analyzed.

b/ Highest and lowest clonal means observed.

c/ Values are percent of seed production capacity.

Extraction efficiencies were generally low (table 3). All orchards had average extraction efficiencies below 95 percent, which is the minimum for good management. However, almost all orchards had at least one clone with an extraction efficiency above 97 percent (figure 1). Harvests are apparently being conducted when the earliest maturing clones are ripe, but at a time before late maturing clones are ready. Close attention needs to be given the

cone ripening date for each clone to assure harvest of only mature cones. This practice will reduce losses caused by casehardening of cones while drying.



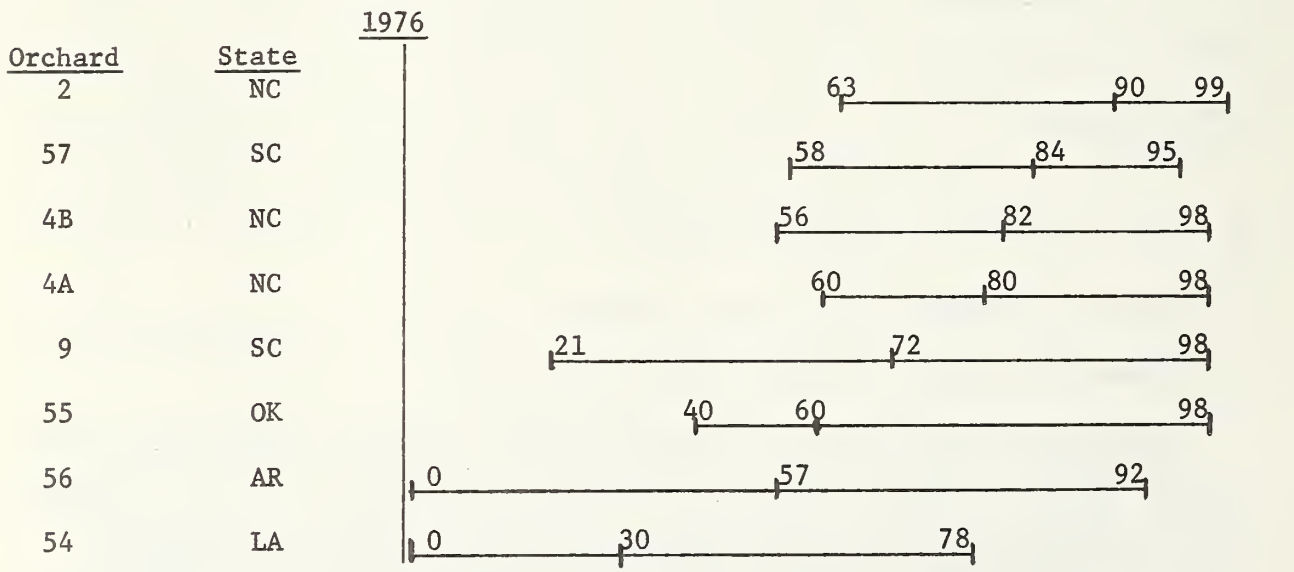


Figure 1.--Loblolly pine extraction efficiencies. Left value is lowest clonal mean in the orchard. Middle value is orchard mean. Right value is highest clonal mean.

Once the earliest ripening clones are ready for harvest, only a short time remains before natural seed fall begins. By leaving late maturing clones to a second pick when they are more ripe, more time would be available for picking all early ripening clones before cones begin opening on the tree and seed is thereby lost. A harvest divided into a picking for early maturing clones and a picking for late maturing clones could, therefore, increase yields from both early and late groups.

The immediate financial consequences of immature cone harvest can be illustrated by a simple example. If only 80 percent of a potential harvest of 1,000 pounds of seed is extracted from harvested cones, 200 pounds of seed are lost. At value on the seed of $100 per pound (Zobel, 1974), this loss would cost $20,000.

Seed production capacities for loblolly seed orchards appear to decrease along a gradient from east to west (figure 2). The highest orchard average in both years was found in an eastern orchard, while the lowest came from a western orchard. However, some western orchards contained clones that had capacities that exceeded the highest clonal averages from some of the eastern orchards. Clonal variation might, therefore, equal or exceed geographic variation. The capacity of each clone to produce should be evaluated. Species or regional means for capacity can serve only as general estimates.

The seed production capacities for other pine species analyzed were: longleaf - 157, slash - 175, shortleaf - 96, Virginia - 84. A detailed discussion on these species is not possible because insufficient data was available on them.

Future Application of SOSET and CAS

Cone analysis provides detailed information, but is restricted in the number of clones to which it can be applied because it is time consuming and costly. SOSET, on the other hand, can be performed relatively quickly and inexpensively, but does not provide as much information as cone analysis. The preferable analysis will depend on the particular problems to be solved or the objectives to be met. A scheme for evaluating and monitoring an orchard for extended and short range planning could be as follows.

An initial step would be a light sampling of all clones in the orchard, primarily to determine seed production capacities by cone analysis. Capacity is a trait under strong genetic control and would not require heavy or repeated sampling for an accurate estimate. Subsequently, or concurrently, a SOSET analysis could be applied to larger samples from each clone to evaluate realized production (seedling efficiency, Karrfalt and Belcher 1977). Poor producing clones could be identified from the SOSET results. These poor clones could then be analyzed more closely by cone analysis to help find ways to improve their performance, or to decide whether or not to replace them with more productive clones (fig. 3). Meanwhile, SOSET can still be applied to monitor any possible changes in production from better clones.

As an alternative to a light cone analysis, seed production capacities could be estimated from the same cone samples used in the SOSET analysis.

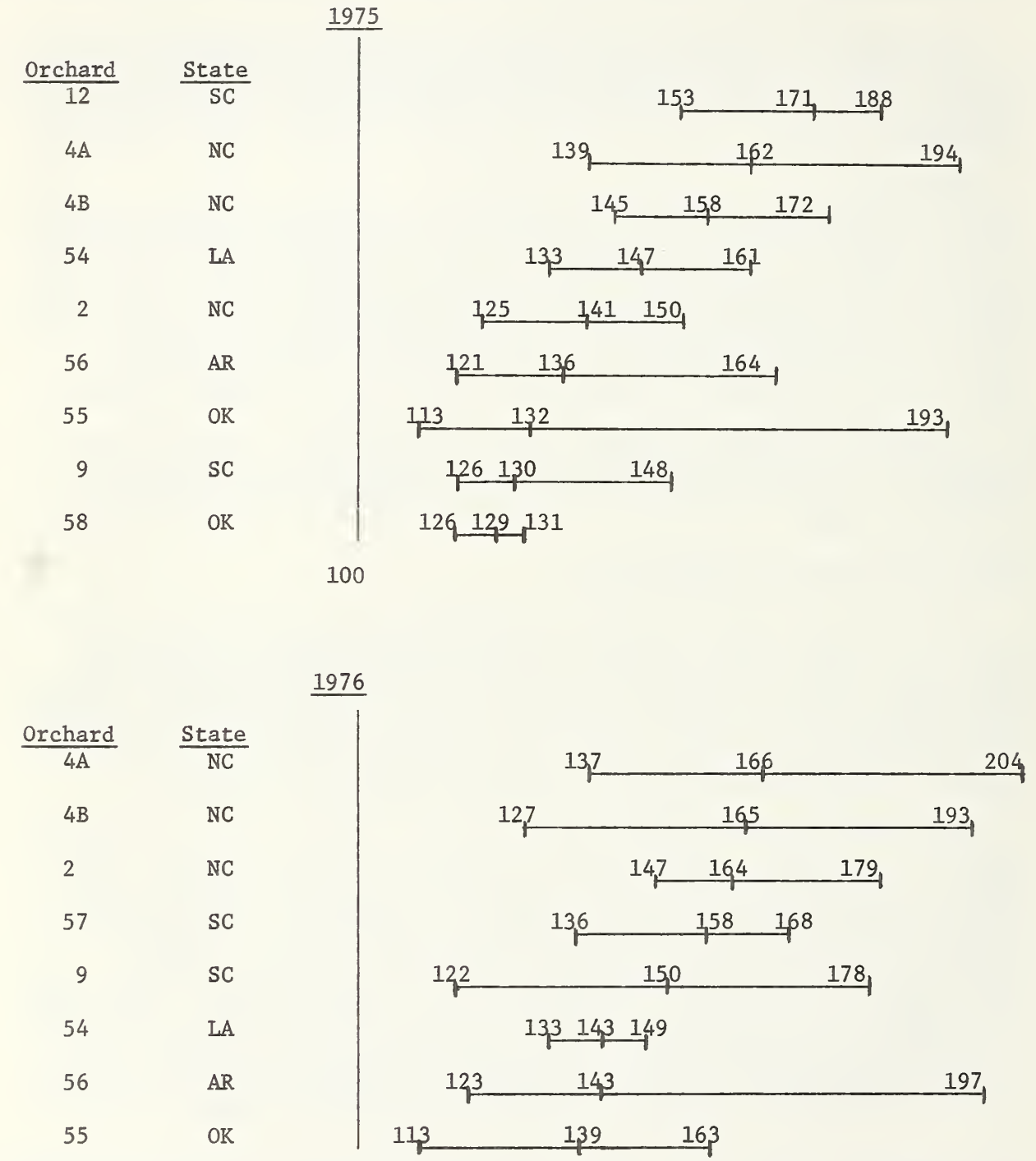


Figure 2--Loblolly pine seed production capacities. Left value is lowest clonal mean in the orchard. Middle value is the orchard mean. Right value is highest.

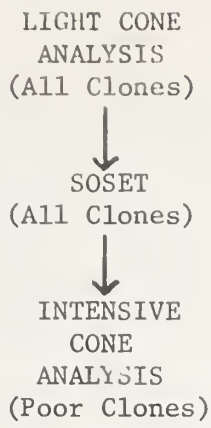


Figure 3.--Scheme for total orchard evaluation

LITERATURE CITED

Belcher, Earl W. Jr. and Hitt, Robert G. 1973. Observations on two-year results of the seed orchard survey (S.O.S.). 12th Southern For. Tree Improvement Conf., Proc. p. 55-63.

Belcher, Earl W. Jr. 1974. Observations on the third-year results of the seed orchard survey (S.O.S.). In Proc. Seed yield from southern pine seed orchards, John Kraus, Editor. Georgia Forest Research Council, Macon, Georgia.

Bramlett, David L. 1974. Seed potential and seed efficiency. In Proc. Seed yield from southern pine seed orchards, John Kraus, Editor, Georgia Forest Research Council, Macon, Georgia.

Karrfalt, R.P. and Belcher, E.W. Jr. 1977. Evaluation of seed production by cone analysis. 24th Northeastern For. Tree Improvement Conf., Proc. 1976. p. 84-89.

Lyons, L.A. 1956. The seed production capacity and efficiency of red pine cones (Pinus resinosa ait.) Can. J. Bot. 34: 27-46.

Zobel, B.J. 1974. Summary comments. In Proc. Seed yield from southern pine seed orchards, John Kraus, Editor. Georgia Forest Research Council, Macon, Georgia.

PITCH CANKER OF LOBLOLLY PINE IN SEED ORCHARDS

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Abstract.--A shoot dieback of loblolly pine (Pinus taeda L.) is endemic in many seed orchards across the South, but in 1975 it suddenly became severe in two loblolly seed orchards. This disease has been identified as pitch canker and the causal organism as Fusarium moniliforme var. subglutinans Wr. & Reink. The pathogenicity of the fungus has been confirmed. Susceptibility among clones varies from apparently immune to highly susceptible. In some clones, 81-100% of the ramets are affected. Disease severity varies from 1-2 affected branches to a killing of more than half the crown. In orchards, symptoms of the disease develop throughout the year. In fall, needles on cankered shoots turn yellow to reddish brown. Buds and expanding new shoots often die and rapidly turn a vivid brown the following spring as the disease girdles the branches. Several environmental and cultural factors that may contribute to disease development are being investigated. Controls have not yet been developed.

Additional Keywords: Fusarium lateritium f. sp. pini, stress, geographic source, fusiform rust, wounds, Contarinia sp.

INTRODUCTION

A disease called shoot dieback has been known by seed orchard managers for at least 15 years (B. Zobel personal communication). Incidence of the disease has waxed and waned in various orchards across the South. Limited attempts by pathologists, entomologists, soil scientists, and tree improvement personnel had failed to identify the cause of the problem.

In early 1971, Howard Johnson of International Paper Company noted a shoot dieback of several loblolly pines in the McNair Seed Orchard near Natchez, Mississippi. An April 1971 survey showed 80 ramets in 17 clones had dieback symptoms. Damage was most prevalent, however, in three clones. When the trees appeared to recover, little attention was paid to the problem, but in 1975 shoot dieback became severe in the McNair Orchard as well as in the Piedmont section of the J. P. Weyerhaeuser Seed Orchard near Washington, N. C. Disease spread was rapid in the McNair Orchard in 1975 and a survey made in July and August indicated that 18% of the ramets were affected. By December, 45% of the trees

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had moderate to severe shoot dieback. The effect on seed production was catastrophic. In 1975 a record 3,428 bushels of cones were harvested at the McNair Orchard. A year later, production was only 467 bushels, largely because of the dieback problem.

At the Weyerhaeuser Orchard a concurrent symptom of severe defoliation in the summer of 1975 greatly increased the dramatic appearance of the dieback. Some trees were so completely defoliated that at least one was erroneously considered dead and so was felled. An April 1976 survey indicated 48% of the trees in the Piedmont section were affected by the dieback. The trees in both orchards now appear to be recovering, but branches and new shoots are still dying, especially on ramets of highly susceptible clones.

Research during the past year indicates that shoot dieback is a disease known as pitch canker. The causal fungus is Fusarium moniliforme var. subglutinans Wr. & Rienk.

LITERATURE

Pitch canker was first observed on Virginia pines (P. virginiana Mill.) in 1946 (Hepting and Roth 1946). The disease occurs on pines from Virginia to southern Florida and West to Mississippi (Hepting and Roth 1953). In 1953, it was reported that loblolly pine was not a host of the fungus which causes pitch canker (Hepting and Roth 1953). However, a separate strain of the fungus was later reported to attack loblolly pine in eastern North Carolina (Hepting 1971). Recent studies show that current isolates of the pitch canker fungus from several pine species are able to attack loblolly pine (Dwinell 1976, 1977).

CAUSAL AGENT

The nomenclature of the fungus that causes pitch canker is being re-evaluated. In 1946 it was determined that the causal fungus was a species of Fusarium, possibly in the section Liseola (Hepting and Roth 1946). However, in 1949, the fungus was named F. lateritium (Nees) emend Snyder and Hansen f. sp. pini Hept. (Snyder, Toole, and Hepting 1949), placing it in a distinctly different section of a very large and diverse genus. Current isolations made from shoot cankers in seed orchards consistently yield a fungus which has been identified as F. moniliforme var. subglutinans, a member of the section Liseola. This species is a pathogen of corn, cereals, and a host of other agronomic crops (Booth 1971). Since Fusaria are ubiquitous as saprophytes, it was thought this species was a secondary invader. However it was subsequently isolated from pitch cankers on slash (P. elliottii Engelm. var. elliottii), South Florida slash (P. elliottii var. densa Little and Dorman), shortleaf (P. echninata Mill.), longleaf (P. palustris Mill.), and Virginia pines (Dwinell 1977).

Because this fungus was isolated from more than 95% of the affected sample branches, pathogenicity tests were undertaken (Dwinell 1976, 1977). Table 1 shows some of the results of inoculating loblolly pine seedlings with isolates identified as F. moniliforme var. subglutinans (Dwinell 1977).

Table 1.--Mortality of new shoots of 1-year-old seedlings of loblolly pine inoculated with isolates of F. moniliforme var. subglutinans from various pine sources (Dwinell 1977).

Pine Source	Number of Isolates	Number of Seedlings Inoculated	Shoot Mortality (Mean % ± s.d.)
Loblolly	27	327	39 ± 27
Slash	16	194	27 ± 18
Shortleaf	6	63	14 ± 14
Virginia	6	58	13 ± 17

A mycological study now underway will characterize the fungus that causes pitch canker. Based on interpretation of the original description of the pitch canker fungus (Snyder, Toole, and Hepting 1949) and pathogenicity studies (Dwinell 1976, 1977), we believe we are dealing with the same fungus that was described in 1949 as the cause of pitch canker.

SYMPTOMS

Shoot dieback in the upper crown is the predominant system of pitch canker in loblolly seed orchards as well as in slash pine plantations in Florida (Dwinell and Phelps 1977). Shoot dieback is prominent in the fall, when the fully developed needles turn yellow to reddish brown. Usually an entire flush is killed back, creating a bright red-brown flag. Close examination of shoots in the upper crown reveal early symptom development. Initially only one or two needle fasicles will be dead. Resin is often exuded. As the disease develops, more needles turn reddish brown; and once the stem is girdled all needles distal to the girdle become dehydrated and die. Spread down the stem appears to be arrested by nodes. In the spring, shoot dieback resumes because some infections near the end of the shoot do not kill the buds. Since the shoot is unable to supply water, the expanding bud dies. The flush may be fully expanded before being killed by the infection on the older tissue. The foliage rapidly changes from green to brown so that new flagging with vivid brown symptoms may seem to occur overnight. In orchards, symptoms occur throughout the year.

The cambium and young phloem in the infected area turn a bright reddish brown. Developing cankers can be located on shoots where one or more needle fasicles are dead and fresh resin is present. Removal of the outer bark at these spots reveals the discolored cambium from which the fungus can be readily isolated.

Dead shoots remain in the crown for several years as indicators of the disease. In more resistant clones only a few dead laterals occur, whereas in highly susceptible clones the upper one-fourth to one-half of the crown is often dead. Small witches' brooms develop in some trees as adventitious buds form in response to repeated infections and diebacks.

The classic symptoms of pitch canker are bleeding resinous cankers on the trunk and larger branches (Hepting and Roth 1946). This type of bole canker, though uncommon on loblolly pine, retains the bark and is slightly depressed. Resin production varies just as it does on affected shoots. The underlying wood usually contains wedges of pitch-soaked tissue. On branches, pitch cankers are frequently associated with cone removal injuries. The degree of pitch soaking of the underlying wood is sometimes quite extensive. The cankers are sunken, but little external resin may be noticed.

GEOGRAPHICAL AND CLONAL VARIATION

There is considerable variation in the susceptibility of loblolly pines to pitch canker. At the Weyerhaeuser Seed Orchard, pitch canker incidence was markedly higher in the Piedmont seed source than in the Mississippi-Alabama, or north and south Coastal Plains sources. At the McNair Seed Orchard the incidence of pitch canker was highest in the seed source from south-central Arkansas and north-central Louisiana (Table 2). However, it is fairly uniformly distributed over all geographic sources.

Table 2.--Incidence of pitch canker at McNair Seed Orchard in 1975 by geographic source.

Geographic Source	Number of Clones	% Ramets with Pitch Canker
E. Texas	1	50.8
S. W. Arkansas	3	33.3
N. W. Louisiana	3	40.6
S. Cent. Arkansas-N. Cent. Louisiana	4	72.5
S. E. Arkansas	2	44.6
Cent. Louisiana	10	40.0
S. W. Mississippi	9	38.6

There is a marked clonal variation in disease susceptibility at both orchards (Table 3). At McNair only one clone from Northwest Louisiana has a history of no pitch canker infection. At Weyerhaeuser, even in the hard hit Piedmont section, six clones have no damage and four others have only minimal damage. Clone 9-18 is highly susceptible at Weyerhaeuser and has also shown symptoms of pitch canker on ramets in orchards at Tillery and Lumberton, N. C. Pitch canker incidence in the other geographic sources at Weyerhaeuser is limited to a few clones.

Incidence of the disease on ramets of some clones at McNair has varied with time. For example, clone HO-8 ranked first in susceptibility in 1971, but dropped to 15th out of 32 clones in 1975. On the other hand, clone OH-7 was 2nd in 1971 and 4th in 1975.

At Weyerhaeuser and McNair the disease is spread randomly throughout the orchard on the susceptible clones. There is no indication that ramets on either the edge or the middle of the orchard are more heavily infected. Variation in incidence is dependent only on the clone.

WOUNDING AND INSECTS

Without wounds F. moniliforme var. subglutinans probably could not attack a host plant. Insect wounds have been suggested as places of infection (Berry and Hepting 1959). Matthews (1962) reported an association of tip moth damage with pitch canker in north Florida slash pine. McGraw et al. (1976) reported no correlation of subtropical pine tip moth (Rhyacionia subtropica Miller) with incidence of pitch canker in central and southern Florida slash pine. Pitch canker damage in seed orchards is occurring on 15 to 20-year-old trees where tip moth damage is not extensive. Needle midge (Contarinia sp.) is common in seed orchards (Overgaard et al. 1976), and causes a needle wound that is colonized by F. moniliforme var. subglutinans. However, we do not know if the fungus is able to grow to the stem from these needle wounds.

Pitch canker is also associated with mechanical wounding. Infections at cone scars in the McNair Seed Orchard have already been mentioned. Bole cankers may be related to damage caused by seed orchard equipment. In slash pine seed orchards, bole cankers are often associated with injuries caused by mechanical shakers used for cone harvest (Dwinell and Phelps 1977).

The pitch canker fungus also attacks fusiform rust galls (Berry and Hepting 1959). The fungus has been isolated from pitch-cankered fusiform rust galls on loblolly pines in Florida and Georgia. The fungus probably enters the galls through insect wounds.

Table 3.--Clonal variation in the incidence of pitch canker in two seed orchards.

% Ramets with Pitch Canker	Number of Clones	
	McNair	Weyerhaeuser[a/]
0-20	6	10
21-40	8	2
41-60	11	6
61-80	3	2
81-100	4	7

[a/] Piedmont seed source.

Stress.--Diseases such as pitch canker are frequently associated with some stress factor, such as a moisture deficiency, early frost, or cultural practice that predisposes the tree to infection. Information provided by the seed orchard managers, however, does not indicate that either moisture deficiencies or early frost are involved in the current outbreaks. Opinions vary widely on what cultural practices might be involved. Experiments with various formulations and concentrations of Guthion[R] did not result in visible phytotoxic symptoms (G. F. Fedde and G. L. DeBarr, personal communication). Fertilization may increase susceptibility by providing a more succulent host or prolonging growth in the fall. In Florida, the incidence of pitch canker on slash pine appeared to be related to heavy applications of fertilizer (Wilkinson, et al. 1977).

CONTROL

Although there is not sufficient research data to recommend control measures at this time, early results on the effect of fungicides and insecticides on disease incidence in slash pine plantations are promising. Since a number of fungicides are effective in controlling other diseases caused by Fusarium spp., control should be possible. Presently, we are also trying to determine when infection occurs so that application of the fungicides can be limited to these times. Carbofuran, a systemic insecticide, was suggested as a control by seed orchardists, but was not effective in one trial (Table 4). The systemic insecticide phorate appeared to reduce pitch canker in slash pine plantations in Florida (Wilkinson et al. 1977).

Table 4.--Effect of carbofuran on pitch canker in McNair Seed Orchard in 1975.

Furadan® Applied per inch DBH (Ounces)	Number of Ramets Treated[a]	% Ramets with Pitch Canker
0	12	33
4	12	33
8	12	25
16	12	25

[a] Six clones; two ramets per clone. Based on southwide Furadan® study established by G. L. DeBarr, USDA Forest Service, Athens, Georgia.

CONCLUSIONS

Pitch canker has become an important tree disease. In addition to loblolly seed orchards, it is heavily damaging slash pine in plantations and orchards (Phelps and Chellman 1976). Pitch canker on loblolly pines has also been found in plantations, on roadside trees, and in residential plantings. Outbreaks on shortleaf pine plantings in Tennessee and a shortleaf seed orchard in Mississippi have also been reported.

The South's tree improvement program could be critically damaged by the increasing presence of pitch canker. Two loblolly pine seed orchards in opposite parts of the South have been severely affected by pitch canker. There is an urgent need to learn more about pitch canker and to find control measures before it invades more seed orchards.

LITERATURE CITED

Berry, C. R. and G. H. Hepting. 1959. Pitch canker of southern pines. USDA For. Serv., For. Pest Leafl. 35, 3 p.

Booth, L. 1971. The genus Fusarium. Commonw. Mycol. Inst., Kew, Surrey, England. 237 p.

Dwinell, L. D. 1976. A shoot dieback of loblolly pine in seed orchards. Proc. Am. Phytopathol. Soc. 3:335. (Abstr.).

Dwinell, L. D. 1977. Susceptibility of southern pines to infection by Fusarium moniliforme var. subglutinans. (Manuscript for Plant Dis. Reptr.).

Dwinell, L. D. and W. R. Phelps. 1977. Pitch canker of slash pine in Florida. J. For. (In press).

Hepting, G. H. 1971. Diseases of forest and shade trees of the United States. USDA Forest Ser. Agric. Handbook No. 386. 658 p.

Hepting, G. H. and E. R. Roth. 1946. Pitch canker, a new disease of some southern pines. J. For. 44:742-744.

Hepting, G. H. and E. R. Roth. 1953. Host relations and spread of the pine pitch canker disease. Phytopathology 43:475. (Abstr.).

McGraw, J. R., R. C. Wilkinson, R. A. Schmidt and E. M. Underhill. 1976. Tip moths and pitch canker in Florida. Inst. Food and Agric. Sci., Univ. Fla. Prog. Rep. 76-1. 2 p.

Matthews, F. R. 1962. Pitch canker-tip moth damage association on slash pine seedlings. J. For. 60:825-826.

Overgaard, N. A., H. N. Wallace, C. Stein, and G. D. Hedet. 1976. Needle midge (Diptera:Cecidomyiidae) damage to loblolly pines in the Erambert Federal Seed Orchard, Mississippi. USDA, For. Ser. SA State & Private Rept. No. 76-213.

Phelps, W. R. and C. W. Chellman. 1976. Evaluation of "pitch canker" in Florida slash pine plantations and seed orchards, 1976. USDA For. Serv., State and Priv. For., Southeast. Area. 22 p.

Snyder, W. C., E. R. Toole, and G. H. Hepting. 1949. Fusaria associated with mimosa wilt, sumac wilt, and pine pitch canker. J. Agric. Res. 78:365-382.

Wilkinson, R. C., E. M. Underhill, J. R. McGraw, W. L. Pritchett, and R. A. Schmidt. 1977. Pitch canker incidence and fertilizer-insecticide treatment. Inst. Food and Agric. Sci., Univ. Fla. Prog. Rep. 77-1. 4 p.

MOVEMENT OF SHORTLEAF PINE SEED ORCHARDS SOUTH MAY GREATLY INCREASE FLOWERING

R.C. Schmidtling [1]

Abstract.--Male and female strobilus production by shortleaf clones of Tennessee-North Carolina origin was evaluated in a North Carolina seed orchard and in a south Mississippi clone bank. Performance of shortleaf clones of Arkansas-Oklahoma origin in an Arkansas seed orchard and in the same south Mississippi clone bank was likewise compared. In both cases the southern location produced five times as many male and female strobili as did the seed orchards located near the origin of the clones even though the clone bank was not managed for seed production. Establishing shortleaf seed orchards south of their origin may be a practical method for increasing seed yields.

Additional keywords: *Pinus echinata*, strobilus, growth, geographic effects.

INTRODUCTION

Many southern pine seed orchards are located in places too hilly for efficient operation of motorized equipment, on seriously deficient sites, or in areas that have unsuitable climates. Deciding where to establish seed orchards in the future is important, and among the elements to consider is geographic location.

Although copious published results of actual experiments are lacking, there seems to be a consensus that moving trees to a warmer climate will probably be desirable (Werner 1975). Recently it has been found that moving seedling slash pine (*Pinus elliottii* Engelm.) south of their natural range can enhance precocious flowering (Gansel 1973). In northern Europe, establishing seed orchards south of their original source is a recommended practice (Sarvas 1970).

This paper reports results of an experiment that tested the effects that southward movement had on the flowering of grafted shortleaf pines (*Pinus echinata* Mill.).

MATERIALS AND METHODS

In 1967 and 1968 the National Forest, Southern Region Tree Improvement Program provided surplus shortleaf grafts from the Ouachita (central Arkansas), Beech Creek (western North Carolina), and the Erambert (south Mississippi) Seed Orchards for establishment in a clone bank on the Harrison Experimental Forest in south Mississippi (fig. 1). In 1976, those clones in the

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[1] Plant Geneticist, Southern Forest Experiment Station, Forest Service--USDA, Gulfport, Miss. I am indebted to the tree improvement personnel of the U.S. Forest Service, Southern Region, for their continuing cooperation in conducting this research.

bank having three or more ramets were paired for measurements with a similar number of ramets of the same clones of equivalent age in the orchards of their origin. Since the clone bank was established using surplus orchard grafts, ramets in the clone bank are identical to ramets in the orchards of their origin with respect to clone, rootstock, grafting technique, scion condition, and early handling.

The clone bank differs from the orchards in two ways. Spacing is 15 x 25 feet in the clone bank, compared with 15 x 30 feet in all the orchards. Since the original purpose of the clone bank was preserving a germ plasm it has not been managed for seed production as the orchards have. Thus, the orchards have been fertilized and had pesticides applied to control insects, but the clone bank has not.

In the spring of 1976, male and female strobili were counted on all ramets. At this time height and d.b.h. were measured on three or four ramets of each clone except those from the Mississippi source which were measured in the fall of 1976. A bulk sample of cones was collected from the clone bank to measure seed yield. In the Arkansas versus Mississippi comparison there were three seed sources with a total of 31 clones; in the North Carolina versus Mississippi comparison, two seed sources with 18 clones; and in the Mississippi versus Mississippi comparison, three seed sources with eight clones (Erambert Seed Orchard versus the clone bank). The last comparison is for inferring site effects only, since 27 miles separated the two plantings (fig. 1). Table 1 summarizes location and establishment details. Although a total of four planting locations are reported here, direct comparison among the three seed orchards is not possible since different seed sources and different clones are used in each case. Each of the orchards can only be compared directly to the clone bank location, though indirect comparisons are possible.

Table 1.--Establishment data for each orchard/clone bank comparison

Orchard location	Age of ramets	Clones	Total ramets	North-South distance to clone bank
	Yrs.	no.	no.	km.	^{o}lat.
N. Carolina	8	18	136	480	4.5
Arkansas	9	31	226	380	4.0
Mississippi	9	8	68	44	0.4

For all pairs of comparisons, the statistical model is:

$$Yijkm = Li + Sj + Cjk + Rjkm + LSij + LCijk$$

where Y is the measured response, L is the location effect, S is the source (provenance) effect, C is clone within source, and R is ramet within clone. Clone and ramet are considered random effects; all others, fixed.

All count data were transformed using $\sqrt{\text{count} + 1}$. Least squares analysis of variance was used for estimating mean squares of treatment effects.

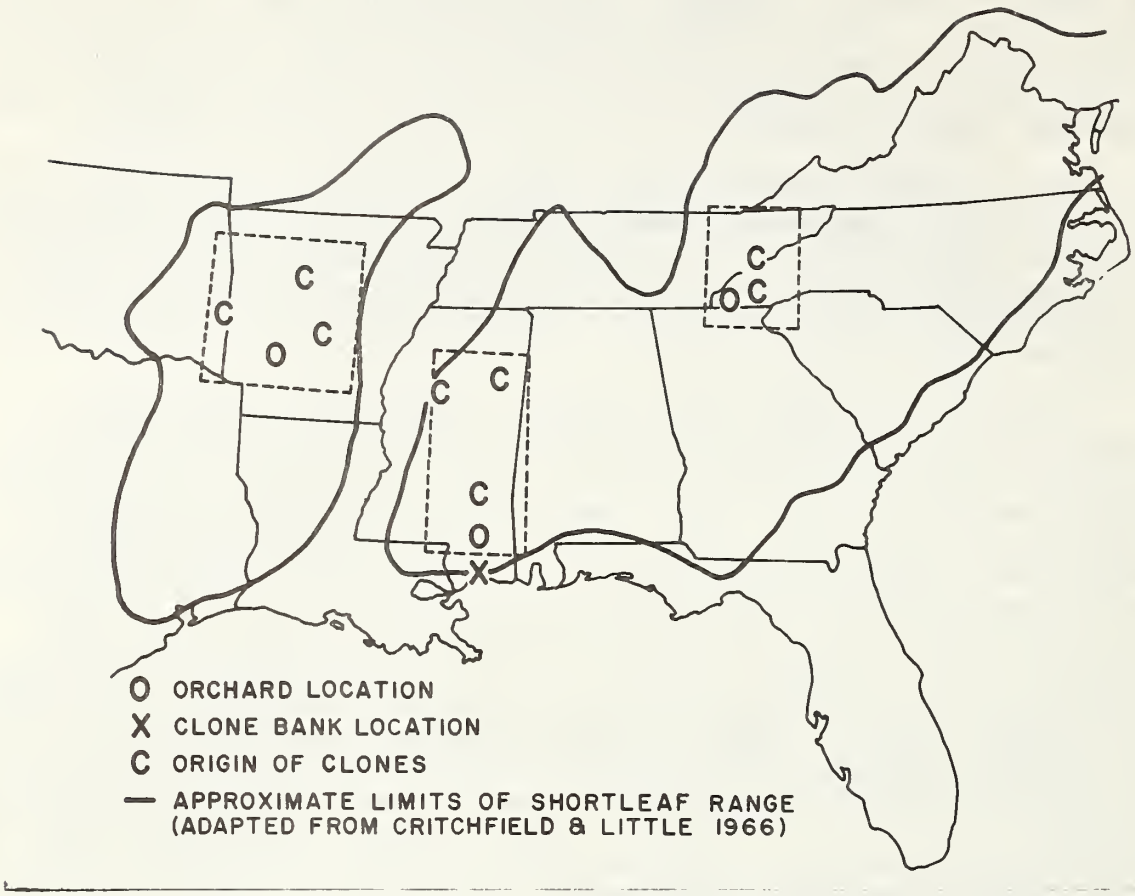


Figure 1.--Location of clone bank, seed orchards, and origin of shortleaf clones.

RESULTS AND DISCUSSION

In both the North Carolina versus Mississippi and the Arkansas versus Mississippi comparisons, female strobili were much more numerous at the clone bank location than at the orchards (fig. 2). In both cases, approximately a fivefold increase was induced by moving the grafts south. The abundance of male strobili is similarly affected (fig. 3); they were about five times more abundant in the clone bank than at the orchards near their origins.

In the statistical analysis, the pattern of significance is similar for male and female flowering for both geographic comparisons. Location and clone effects and their interaction are always significant; geographic source of the clones and its interactions with other effects are not. The interaction was primarily one of ranking, that is, most clones flowered better at the southern location, but the response varied by clone. In some cases, there were fewer strobili in the southern location. Four of the total of 49 clones had fewer female flowers and two had fewer male flowers in the clone bank. The differences were very small, however, and statistically significant in only one instance.

In both comparisons the trees in the clone bank were slightly larger

though probably not enough to explain the differences in flowering. Ramets in the Arkansas orchard trees averaged about 12 feet versus 13 feet tall for the same clones in the clone bank; the North Carolina ramets averaged 11.6 feet versus 12.2 feet for their southern counterparts. Differences in d.b.h. were less striking, and the trees from the central Arkansas source averaged slightly less in d.b.h. at the clone bank than at the orchard.

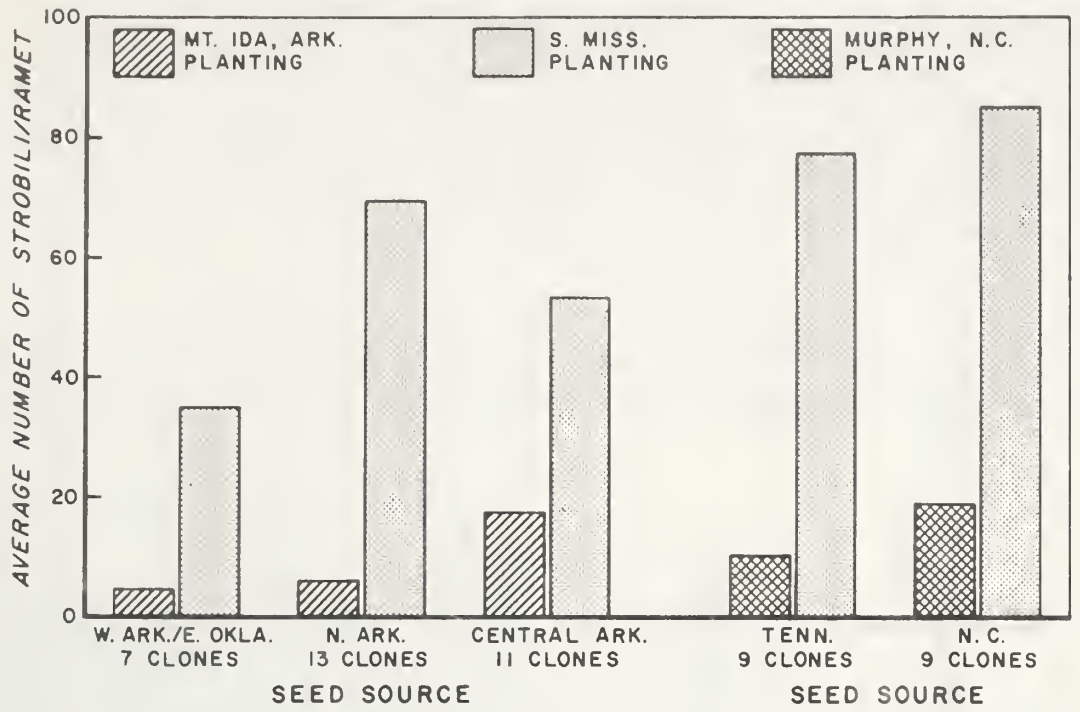


Figure 2.--Female strobili on shortleaf grafts planted near their origin compared with grafts of the same clone planted in a southern location.

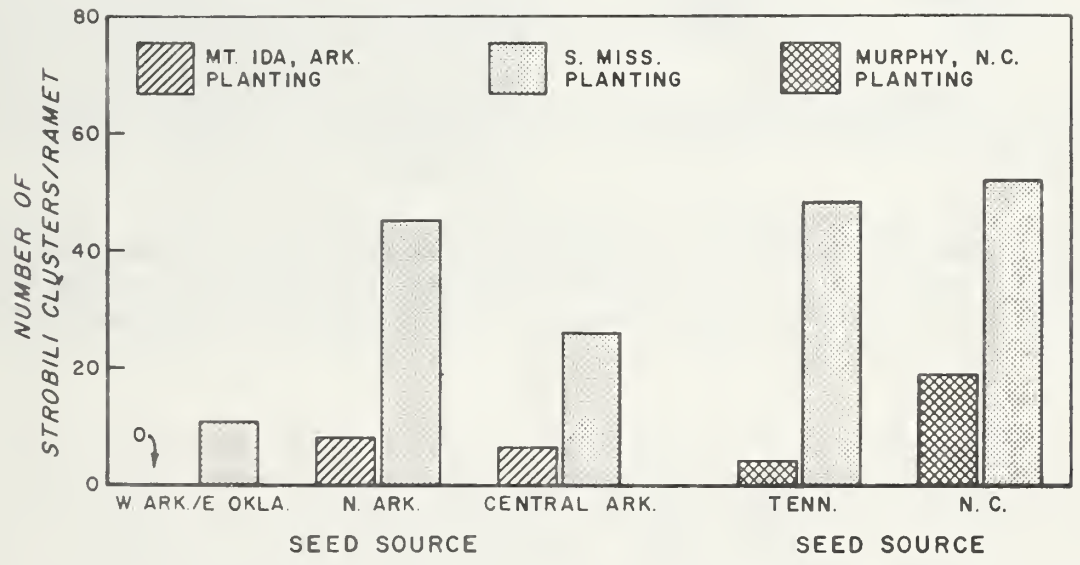


Figure 3.--Male strobili on shortleaf grafts planted near their origin compared with grafts of the same clones planted in a southern location.

Since there are only two locations in each comparison, geographic effects are confounded with site and management differences. There is one comparison, not previously discussed, which illustrates the importance of these differences. Flowering was much better in the clone bank planting than at the northern locations, but the shortleaf pines at the Erambert Seed Orchard, located just 27 miles north, outperformed those in the clone bank. The clones from Mississippi had about six times as many female flowers and about five times as many male flowers at the seed orchard as in the clone bank (fig. 4). Since the sites are otherwise very similar, this difference undoubtedly reflects the difference in management, specifically the fertilization and insect control regimen applied in the orchard. The ramets at the Erambert Seed Orchard are not significantly taller (20.3 feet versus 19.6 feet) but are considerably larger in d.b.h. (4.7 inches versus 3.5 inches) than those in the clone bank.

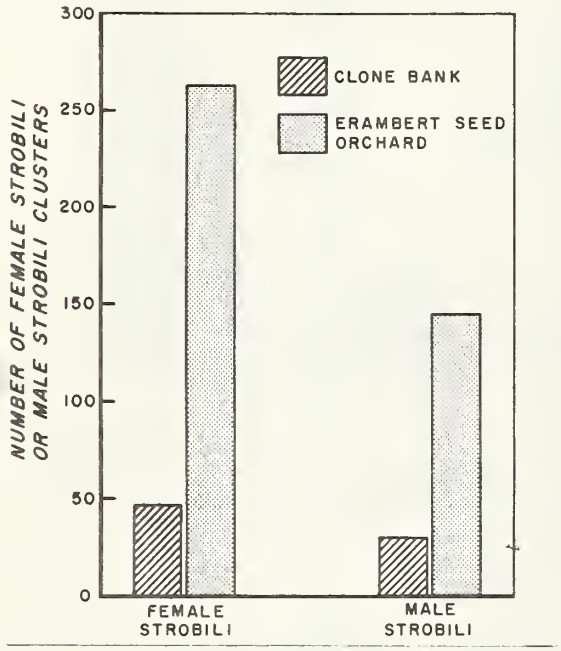


Figure 4.--Flowering in the clone bank compared with the Erambert Seed Orchard.

It is tempting to extrapolate a southern versus northern orchard comparison from our orchard versus clone bank comparisons. The Mississippi and the Arkansas clones are the same age and had approximately equivalent numbers of female flowers in the clone bank (the planting design permits this comparison). The Arkansas clones had five times as many female strobili in the clone bank as in the Arkansas orchard, and the Mississippi clones had six times as many female strobili in the Mississippi orchard as in the clone bank. Thus, one might expect 5 x 6 = 30 times as many female strobili if Arkansas clones were planted in the south Mississippi orchard. While this extrapolation has obvious flaws, site effects would bias the results in the opposite direction from that observed.

The cones collected in the clone bank yielded only an average of 11.6 seeds per cone, of which only 2.5 were filled. A sample taken at the orchard in North Carolina showed approximately 40 sound seeds per cone where insecticide was used, but only three or four per cone where no insecticide was used.[2]

The later figures are comparable to those from the clone bank, as are the probable conditions that precipitated the low yield--the lack of protection from seed and cone insects. In a shortleaf provenance test located about a mile from the clone bank, seed yields were good in controlled crosses involving a wider sampling of the shortleaf range than reported here

[2] Ed Manchester, Manager, Beech Creek Seed orchard, Murphy, N.C. personal communication.

(unpublished data). In another study conducted in this planting, differences in reproductive phenology were so small that none of the sources were reproductively isolated from each other or the local source (Schmidtling 1971).

CONCLUSION

This study appears to lend support to earlier suggestions that southward movement will increase seed orchard productivity. The results reported here are clearcut, but are based on only one year's flowering data and inconclusive seed yield information. Site effects, primarily those related to management, appear to be important. The clone bank will be fertilized to correct this for future work. More importantly, because flower crops vary greatly in a given year from location to location because of variation in local weather conditions, several years' data will be necessary to draw firm conclusions. Nevertheless, I do not anticipate problems involving seed yield arising as a result of movement of shortleaf to the southern edge of its range. A comprehensive survey of southern seed orchard yields is being conducted now by a subcommittee of the Southern Forest Tree Improvement Committee. The survey's results can be used in planning the location of definitive experimental plantings to determine where future orchards should be located.

LITERATURE CITED

Critchfield, W. B., and E. L. Little, Jr. 1966. Geographic distribution of the pines of the world. USDA Misc. Pub. 991, 97 p.

Gansel, C. R. 1973. Should slash pine seed orchards be moved south for early flowering? In Proc. Twelfth South. For. Tree Improve. Conf. p. 310-316.

Sarvas, R. 1970. Establishment and registration of seed orchards. Folia Forestalia 89. Institutum Forestale Fenniae. 24 p.

Schmidtling, R. C. 1971. Geographic races of shortleaf pine not reproductively isolated in a mixed plantation. In Proc. Eleventh For. Tree Improv. Conf. p. 212-217.

Werner, M. 1975. Location, establishment and management of seed orchards. Pages 49-57 in R. Faulkner, ed. Seed orchards. For. Comm. Bull. 54, London: Her Majesty's Stationery Office.

CROWN SHAPING IN A SLASH PINE SEED ORCHARD

Charles R. Gansel[1/]

Abstract.--A seed orchard of high-gum-yielding slash pines was established for seed production and demonstration purposes in 1957 near Lake City, Florida. A tree shaping (pruning) study was imposed at age 9. Pruning decreased cone production slightly but limited height growth for ease of cone collection. Cone production for the first 10 years was generally curvilinear, rising with age. Good seed crops occurred every other year. Tree shaking removed only about 60 percent of the cones on both pruned and unpruned trees. Insect damage to cones and inbreeding effects are also discussed.

Additional keywords: Seasonal trends, tree shaker efficiency, cone insect impact, inbreeding, Pinus elliottii, pruning.

In 1957, a clonal seed orchard of high-gum-yielding slash pines (Pinus elliottii Engelm.) was established near Lake City, Florida to demonstrate orchard management and to provide seed for commercial production of high-gum-yielding trees. In April of 1966, a tree shaping (pruning) study was implemented at this orchard. The objectives of pruning were: (1) to increase cone production by increasing the number of cone-bearing branches, and (2) to keep the trees at a manageable height for easy cone collection. Pruning results and other observations which may be helpful to orchard managers are presented herein.

MATERIALS AND METHODS

The McColskey Still seed orchard was designed to produce third generation seed and contains only 9 clones, all of which are related as half- or full-sibs. It was established in 1957 after 3 of the best gum producing families growing in a 10-year-old progeny test were evaluated, and 3 high-gum-yielding individuals from each family were selected from them for cloning. Airlayers and grafts were planted at a 30 x 30 foot spacing with ramets of a clone no closer than 90 feet. The orchard was mowed, irrigated, and fertilized to promote flowering and growth. The lower third of the crown was removed in 1963 to facilitate mowing.

The pruning study consisted of 4 blocks, with 4 trees of each of the 9 clones in each block. Each of the 4 trees of a clone in a block received a different treatment. Treatments consisted of:

1/ Plant Geneticist, Southeastern Forest Experiment Station, USDA Forest Service, Olustee, Florida.

1. No pruning.
2. Terminal and lateral branches bud pruned.
3. Terminal and laterals pruned back to established branches (heavy pruning).
4. Visual pruning, a combination of the other two pruning methods as the pruner chose.

When the pruned trees exceeded 30 feet in height the tops were sheared back to 30 feet and allowed to increase in height one whorl per year.

Pruning was started when trees were approximately 20 feet tall. Treatments 2 and 4 were carried on every year for 4 years (1966-1969). Pruning of laterals in treatment 3 occurred in 1966 and 1968. The 1966 pruning shaped the bottom portion of the crown while the 1968 pruning shaped the upper portion. After the trees were shaped, only the tops were cut back for an additional 4 years (1970-1974). The time required for pruning and cone collection was recorded. Cone counts were made for 10 years (1966-1975).

RESULTS

Pruning Study

The cumulative time (in minutes) required to shape the treatment trees over the 4-year pruning period was as follows: control - 0; bud pruning - 32; heavy pruning - 20; pruner's choice - 21. The additional top pruning carried on from 1970 through 1973 required approximately 4.6 minutes per tree per year.

The number of cones collected per minute was basically the same for all treatments, but increased with increasing size of crop (table 1). Clonal effects were strong with values ranging from 2.3 cones/min. to 27.2 cones/min. Before tree height became a critical factor in cone collection time, the timing experiment was completed. The controls are now reaching a height where some cones will be uncollectable with present equipment. However, pruned trees on the average are approximately 15 feet shorter and are more accessible than unpruned trees.

Table 1.--Average number of cones per minute collected with a bucket truck and 3 man crew.

	Year of collection							
Treatment	1966	1967	1968	1969	1970	1971	1972	1973
Control	5	9	10	9	8	16	11	14
Bud pruning	5	12	9	7	7	18	9	13
Heavy pruning	4	10	9	6	7	16	10	13
Pruner's choice	5	9	8	8	7	17	10	13
Average number of cones per tree	3	10	19	44	29	187	85	175

Overall pruning had a negative effect on cone production, but differences were not statistically significant over the 10 years (figure 1). Differences in cone production between pruned treatments were generally very small.

Pruning greatly increased the crown density (figures 2 and 3). The differences in crown development were due to pruning. Note the thin foliage at the base of the crown in figure 2. Natural pruning usually occurs at the base of the crown at an early age even when slash pine is open grown. The greatest change occurred in the unpruned trees when the tops were sheared back.

We expected the pruning of the lateral branch tips to increase the number of cone bearing branches, and the top pruning to cause the smaller branches to become larger and better able to bear cones. Branches with a larger diameter tend to have more cones per branch. Tertiary branches were 25 percent larger in pruned trees than in unpruned trees (Varnell 1969).

There was no insect control in the orchard. An insect impact study in the orchard indicated that over half of the flower buds are lost before harvest due to cone aborting insects mainly thrips (Fatzinger, et al. 1975). There is a strong indication that pruning of branch ends attracts these insects. In the upper portion of the crown of pruned trees, 55% of the conelets matured into cones, while 71% matured on the unpruned trees. Pruning results might have been different if insects had been controlled.

Other observations

Cone production cycles.--Cone production in the orchard began in 1966 and followed a rising curvilinear pattern as the orchard became older. The pattern of overall cone production is illustrated by the control (figure 1). Cone production fluctuated annually with relatively good cone crops produced every other year.

Tree shaker collection.--In 1974 and 1975 a shockwave tree shaker was used to remove the cones. The shaker was as efficient with pruned trees as with unpruned ones, but it harvested only 60 percent of the cones in both cases. The efficiency of the harvest varied strongly by clones; the clonal means varied from 37% to 80%.

Insect damage.-- The cones from 2 unpruned ramets per clone were analyzed from 1971-1975. Generally, cone deforming insect infestations follow cycles. In this orchard, cone infestation from 1971-1974 was on a downward trend, but 1975 brought on a large increase (table 2).

Overall, insect infestations of cones by cone moth (Dioryctria) have been relatively low (7.4%) in this orchard compared to other orchards (16%) with no insect control. Seed orchard sanitation may have something to do with low infestation, as all cones have been removed from the area for the past 10 years. High-gum-yielding clones in a clone bank where the cones were not removed had much lower sound seed counts (11 vs. 46) in 1976.

Figure 1.--Average number of cones per tree for various pruning treatments.

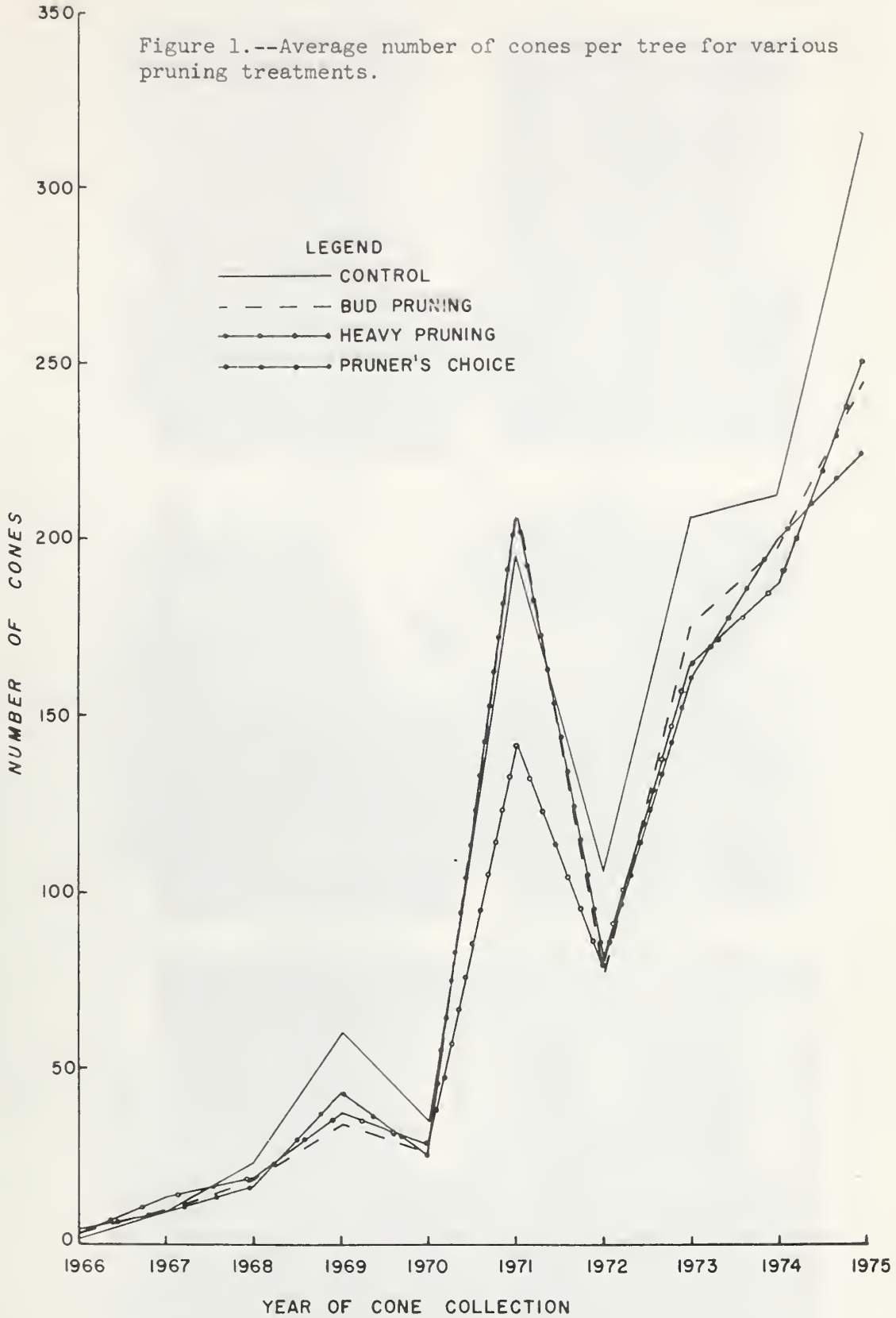
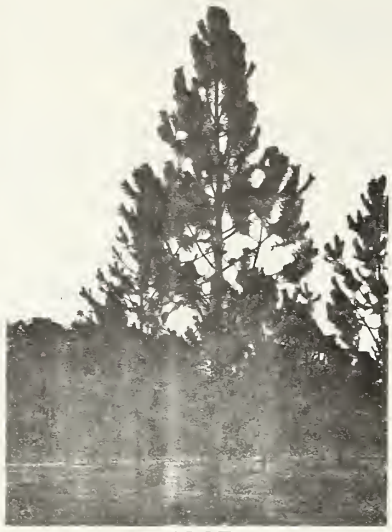


Figure 2.- Normal Development of Narrow-Crowned Tree in Seed Orchard

1966

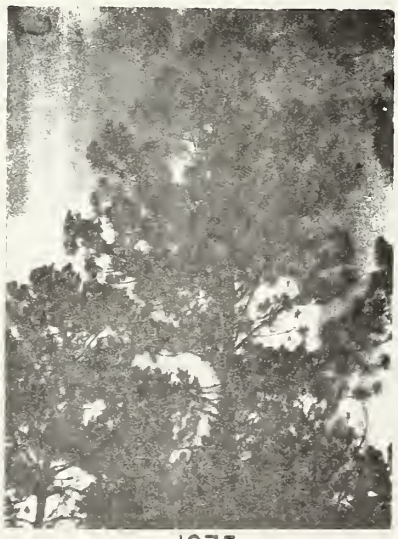


1967

1969

1971

1973



1975

Figure 3.-- Development of Narrow-Crowned Tree with Heavy Pruning in Seed Orchard

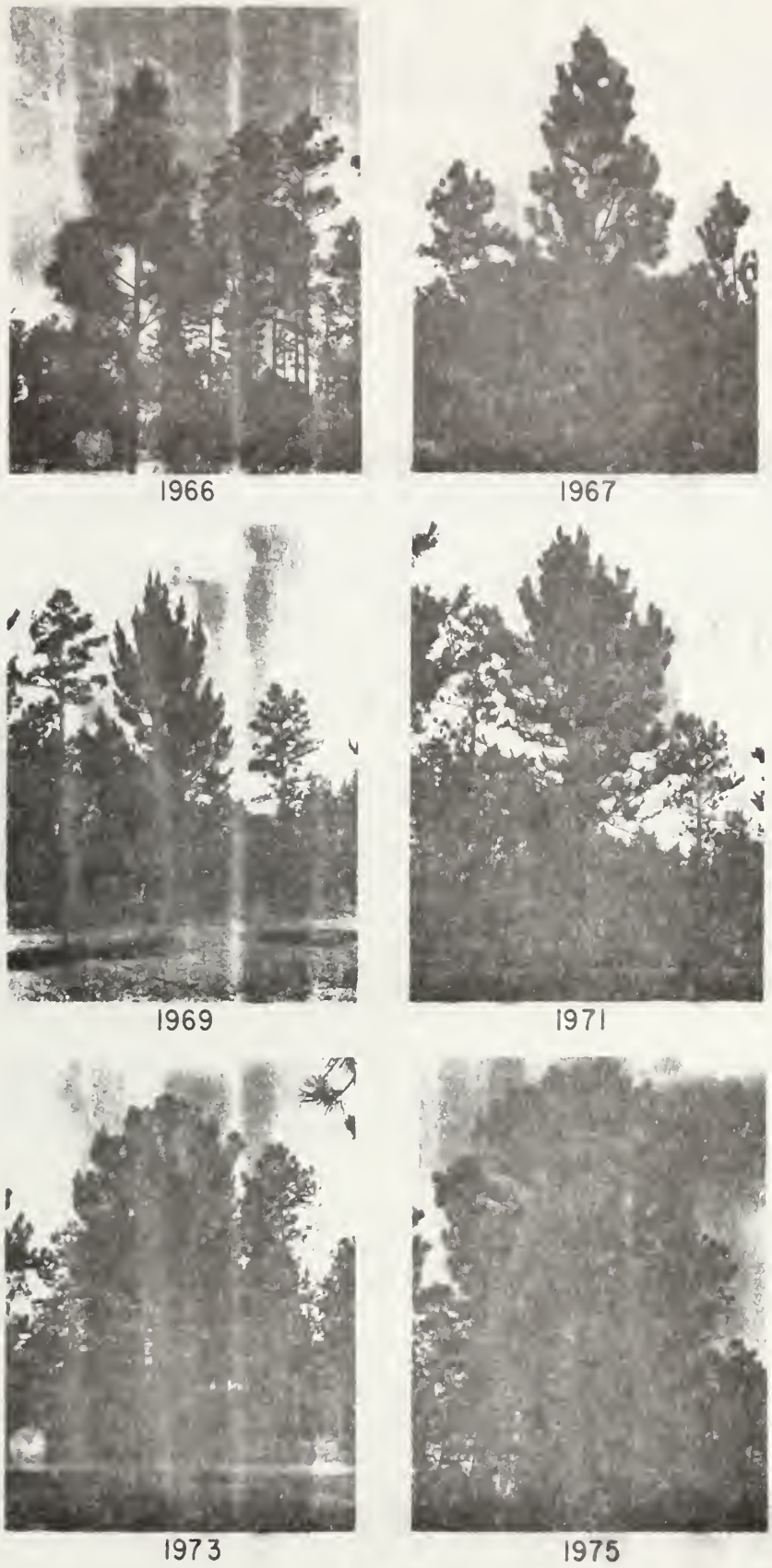


Table 2.--Percent of cones infested with insects on sample trees in McColskey Still seed orchard.

Year	Cones infested %
1971	7.5
1972	4.9
1973	4.3
1974	3.8
1975	14.6

Apparent lack of inbreeding effect on cone production.--Surprisingly, inbreeding seemed to have little effect on this orchard. According to an Eastern Tree Seed Laboratory (SOS) report, the McColskey Still seed orchard produced 23 thousand viable seed per tree in 1971, as compared with an average of 11 thousand for 29 southern orchards tested that year. In short term progeny tests, seedlings grown from seed of this orchard grew to an average height of 10.5 feet in three years; 6% taller than the controls. When tested a second time they averaged 10.9 feet, 20% taller than controls.

Normally inbreeding causes a depression in the growth rate. Yet, the growth data for this orchard indicates that little inbreeding occurred, possibly due to high pollen contamination (Squillace 1967, 1977). An estimate of the inbreeding coefficient (F) for the F_2 progenies would be 0.167 in this orchard, assuming random mating among clones, equal ramets per clone, no selfing, and no outside pollen contamination. Inbreeding depression for height growth should be about 8% for this degree of inbreeding.

CONCLUSIONS

In this study, pruning reduced cone production slightly, but facilitated cone collection by limiting the height of trees. If seed orchard insects had been controlled, the effect of pruning on cone production might have been quite different.

Cone production increased sharply after the 14th year. Three clones did not start significant cone production until their 15th year. The overall trend of cone production, should be of value to tree improvement program managers for projecting orchard yields.

The efficiency of the tree shaker leaves much to be desired. We observed no difference in "shakeability" of pruned or unpruned trees. The timing of the shaking seems to be important. When it is necessary to collect all the cones in the seed orchard, a bucket truck and punch poles should follow the tree shaker. Top pruning then becomes an important factor in seed orchard management, as it can extend the period of efficient cone collection in the orchard.

The fact that only 9 clones were used in the orchard and that they were all related did not seem to decrease seed production or growth of their progeny. However, use of a few clones and/or many highly related clones is not recommended because of the restriction on the genetic base, which in turn may greatly decrease possibilities for future genetic improvement.

LITERATURE CITED

Fatzinger, Carl W., Hertel, Gerard D., Merkel, Edward P. and Lloyd, F. Thomas. 1975. Developing a sampling method to determine effects of insects on yields in southern pine seed orchards. Proc. 13th South. For. Tree Improv. Conf. pp. 127-128.

Squillace, A. E. 1967. Effectiveness of 400-foot isolation around a slash pine seed orchard. J. For. 65(11); 823-824.

Squillace, A. E. 1977. Monoterpene composition of cortical oleoresin in Pinus elliottii and its utility in genetics research. Paper presented at EEC Symposium on Forest Biochemistry, Brussels, Belgium. Jan. 1977.

Varnell, R. J. 1969. Female strobilus production in a slash pine seed orchard following branch pruning. Proc. 10th South. For. Tree Improv. Conf. pp. 222-228.

EFFECTS OF FERTILIZATION ON SEED ORCHARDS

Jack T. May[1/]

Abstract.--The effects of fertilization on five Forest Service seed orchards in the southern region were variable between and within orchards. Fertilization stimulated the establishment and growth of the vegetative ground cover and the trees. Cone production was significantly correlated with clones, size of trees, cycles, and fertilizer treatments. Moderately high levels of N (224 to 364 kg/ha) and P (97 to 158 kg/ha) generally gave the best yield of cones. High levels of P (195 kg/ha) and K (373 kg/ha) tended to reduce cone production. Low levels of N (84 kg/ha), P (37 kg/ha) and K (70 kg/ha) did not stimulate production.

Additional keywords: Fertilization, cone production, Pinus taeda, P. echinata.

Many treatments and techniques have been tried and used to stimulate flower and seed production in southern pine seed orchards, [Barnes and Bengtson (1968), Green and Taylor (1974); Fuentes (1969); Long et. al. (1974); Pritchett (1967); Schmidtling (1969, 1971, 1975); Schultz (1971); Schultz et. al. (1975); Van Buijtenen (1965); Varnell (1976)]; but results have been extremely variable and indicate that special treatments may be needed for specific species, areas and situations.

This paper discusses the effects of fertilization on grass cover, tree growth and cone production on five U. S. Forest Service seed orchards established in 1961 on diverse sites with low inherent fertility in North and South Carolina, Mississippi, Louisiana and Arkansas. Most of the planting and/or grafting was complete by 1975.

DESCRIPTION OF ORCHARDS

Francis Marion Seed Orchard

This Orchard is located in the coastal flatwoods on the Witherbee Ranger District, Francis Marion National Forest in South Carolina. The site had been stocked with a second growth stand of predominately loblolly pine (Pinus taeda L.) and longleaf pine (P. palustris Mill.) which was clear-cut. The landscape consist of broad, nearly level ridges with very gentle slopes and depressions with the maximum slope < 2 percent. The drainage classes range

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from very poorly drained to well drained. Soils include Bayboro and Caroline fine sandy loam, Duplin and Dunbar sandy loam, Goldsboro loamy sand and Coxville loam. The sub-soil ranges from sandy clay loam to clay with mottling at 48 to 64 cms. Drainage was a major problem affecting orchard management as the water table was within 1.2 m for all series except the Caroline fine sandy loam. Due to the nearly level soil surface and depressional areas, natural drainageways could not remove excess rainfall within a reasonable time and water percolation through the soil was very slow due to the soil texture and structure.

Twenty-one soil samples taken in 1965 from 10 to 15 cm depth showed a mean and range as follows: pH - 5.0 (4.4 to 5.2); P - 1.5 ppm (0 to 8); K - 21 ppm (10 to 31); Ca -907 ppm (220 to 1230) and Mg - 13 ppm (4 to 30).

Beech Creek Seed Orchard

The orchard is located on the Tusquitee Ranger District, Nantahala National Forest in the Blue Ridge Mountain Province of North Carolina. Much of the area was cleared during the early nineteenth century and converted to fruit orchards and pasture. Late in the 19th century the land reverted back to a mixed forest. The landscape consists of ridgetops, gentle to steep slopes and floodplains. Soils developed in materials of the Great Smoky conglomerate geologic formation consisting of graywacke sandstone and conglomerate with interbeds of slate. The upland soils are well drained fine sandy loams to stony fine sandy loam, in the Hayesville series. Slopes range from 2 to 45 percent. The subsoil is a clay loam to clay. Soils are highly erodible. Soils of the coves, toeslopes and bottomland floodplains are in the Brevard, Cartecay, Colfax and Wehadkee series. Eighteen soil samples from the uplands taken in 1963 showed a pH of 5.1; P - Very low to low; K - Low to medium; Ca - Low and Mg - Very low. In 1967, after liming and fertilization 17 soil samples show a mean and range as follows: pH - 5.7 (5.5 to 6.0); P - 14 ppm (5 to 36); K - 32 ppm (20-48); Ca - 248 ppm (151-343); Mg - 41 ppm (27 to 61).

Erambert Seed Orchard

The orchard is on the Black Creek District, Desota National Forest in the Middle Coastal Plain of Mississippi. Most of the area was forested with pole-size loblolly and longleaf pine. Soils developed in materials of the Citronelle formation consisting of white clay and red sand containing gravel. Upland series are well drained Iuka, Lucy, Ruston, Vaucluse, Norfolk and Orangeburg loamy sands, and Orangeburg and Vaucluse stony loamy sand. Subsoil ranges sandy clay loam to clay loam. Soils are highly erodible.

The landscape varies from gently rolling to hilly and steep, with slopes from 2 to 17+ percent. Seven soil samples collected in 1963 show a mean and range as follows: pH - 5.3 (5.1 to 5.5); P - 1 ppm (0 to 4); K - 35 ppm (20 to 64); Ca - 152 ppm (74 to 260); Mg - 38 ppm (27 to 53).

Stuart Seed Orchard

The orchard is on the Catahoula Ranger District, Kisatchie National Forest in the Coastal Plain Province of the Gulf Coast in Louisiana. Most of the site was covered with sedges (Carex spp) and bluestems (Andropogon spp) with scattered pulpwood and post size loblolly and longleaf pine at the time the orchard was established.

Soils developed from undifferentiated clays, sands, sandstone and volcanic ash. The landscape ranges from nearly level to rolling with slopes up to about 12 percent. Soil series of ridgetops, upland flats and slopes are Bowie, Beauregard, Muskogee and Acadia silt loams to fine sandy loams. The subsoils are silt loam to clay with mottles starting between 38 and 76 cm. Drainage varies from well to somewhat poorly drained. Soils are highly erodible. Soil analysis prior to 1973-74 are not available. Samples collected in 1973 from areas that received a light fertilization showed a mean as follows: pH - 5.5; P - < 3 ppm; K - < 24 ppm; Ca - 384 ppm; Mg - 36 ppm.

Ouachita Seed Orchard

The orchard is located on the Womble Ranger District, Ouachita National Forest in Arkansas. The site was stocked with a mixture of shortleaf pine (P. echinata Mill.) and mixed hardwoods. Soils formed in weathered material from shale. Hard shale bedrock is within 0.6 to 1.5 m of the surface in most locations. Limestone, calcite and quartzite occurs in 0.5 to 5 cm stratified layers. The landscape varies from undulating with shallow depressions to hilly. The slope gradient averages about 10 to 12 percent with extremes of 30+ percent. Soils of the upper slopes and ridges are Goldston shaly silt loam, and Herndon gravelly silt loam. Altavista, Wickham, Lindside and Foley series have developed on low stream terraces and alluvial bottoms. Goldston and Herndon series are well drained with medium to rapid run-off. A 1965 soil analysis of two samples showed: pH - 4.6 to 4.8; P - 2 to 5 ppm; K - 42 to 51 ppm; Ca - 280 to 320 ppm; and Mg - 45 to 55 ppm.

EARLY MANAGEMENT OF ORCHARDS

Site preparation, i.e. clearing, grading, disking, fertilization and seeding of a ground cover was completed for a major portion of the Orchards between 1962 and 1965. Species used for ground cover included: bahiagrass (Paspalum notatum), bermudagrass (Cynodon dactylon (L.) Pers.), fescuegrass (Festuca arundinaceae Schrab.), lovegrass (Eragrostis curvula (Schrad.) Nees), browntop millet (Panicum pamosum), sudax (sorghum spp.), and some clovers (Trifolium spp.) and lespedezas (Lespedeza spp.)

Early fertilization of the ground cover was based on recommendations of state soil testing labs--with some variations within orchards. Fertilizer rates were: Francis Marion: 2240 kg of lime, 1344 kg of 4-12-12 and 112 kg of 21-53-0 per ha; Beech Creek: 84 kg/ha of 8-8-8; Erambert: 336 kg/ha of 10-20-10 or 13-13-13; Stuart: 2240 kg of lime and 258 kg of 10-10-10 per ha; Ouachita: 2240 kg of dolomitic limestone and approximately 560 kg of 13-13-13 per ha.

Planting of root stock and/or transplanting of ramets began in 1963 and continued until 1975. When ramets were moved from the nursery or greenhouse to the orchard, 227 to 453 g of 10-10-10 or 12-12-12 were applied in the hole. Maintenance fertilization at rates of approximately 224 to 336 kg/ha of 10-10-10 was applied at 1 to 4 year intervals between rows of trees. In either 1968 or 1969 individual trees were fertilized at a rate of 453 g of 13-13-13 per 2.54 cm of dbh.

Problems that appeared in the various orchards during the early years were: 1) injury to ramets due to excessive fertilization; 2) poor drainage and soil compaction on the Francis Marion Orchard; 3) erosion on the Erambert and Stuart Orchards; 4) poor drainage and very slow tree growth on the Stuart; 5) an invasion of native grasses and weeds.

Because of problems in the orchards the U. S. Forest Service, the University of Georgia and the Georgia Forest Research Council entered into an agreement in 1967 which provided that the University and the Council provide on-the-site consultation for soil related management problems in the seed orchards. Immediate actions included: 1) a intensive soil map of each orchard by The U. S. Forest Service soil scientists; 2) chemical analysis of soil samples that were collected from selected areas in each orchard; 3) correction of drainage problems in the Francis Marion and Stuart Orchards; 4) control of erosion in the Erambert and Stuart Orchards; and 5) improvement of the ground cover by an increase in fertilization and control of pest plants.

FERTILIZATION TO STIMULATE CONE PRODUCTION

In 1971 a fertilization study to stimulate cone production was initiated in each orchard as many of the clones were producing female flowers and stems diameters of the larger trees were 10 cm dbh or larger.

Procedure

A statistical response surface design was used because it was easier to fit than a complete factorial; and it tends to locate the combination of values for the controllable factors (N, P and K) that optimize the response (Cochran and Cox, 1957; Clutter, 1968). A central composite second order design in three incomplete blocks with 3 $\bar{x}$-variables provides 20 treatment combinations.

Treatment levels per ha were: N - 0, 84, 224 and 364; P_2O_5 - 0, 84, 224, 364 and 448; K_2O - 0, 84, 224, 364, and 448. The 448 kg/ha rate of N was inadvertently omitted.

In each orchard, there were 50 clones in each row. Spacing of ramets was 4.5 m within rows and 9.14 m between rows. One set of three incomplete blocks contained 20 rows of trees. Fertilizer treatments were applied in bands approximately 1.8 m wide on each side of a row of trees. Treatments were scheduled for February or March of each year. Cones were collected and counted in the fall of each year. Number of trees for each orchard were as follows: Francis Marion, Beech Creek and Stuart - 1000 each; Erambert - 2000; and Ouachita - 5000.

RESULTS

Ground Cover and Tree Growth

The ground cover, especially seeded grasses, responded readily to fertilization. Visual observations indicated that the density and height of grasses

increased with increasing rates of nitrogen and phosphorus. Erosion was controlled as the grass cover was stabilized.

All levels of fertilization increased diameter growth of trees. D.B.H. measurements were obtained for all trees in the Beech Creek Orchard Study. Trees in the Erambert, Stuart and Ouachita Orchards were grouped into two size classes in 1974-75, i.e. < 9 cm and > 9 cm; but in 1976, trees in the Ouachita orchards were grouped into three size classes, i.e. < 9 cm; 9 to 14 cm and > 14 cm.

The mean dbh's of the Beech Creek Orchard ranged from 8.4 cm for treatment 6 (84-84-364 kg/ha of N, P_2O_5 and K_2O) to 12.4 cm for treatment 12 (364-364-84 kg/ha of N, P_2O_5 and K_2O). Mean dbh for the control plots was 7.4 cm. Percent of larger trees (sizes 2 and 3) was almost consistently higher for treated plots as compared with controls (Table 1).

Table 1.--Percent of size 2 and 3 trees for treated plots and the control.

Orchard	Size 2 trees (> 9 cm)		Size 3 trees (> 14 cm)	
	Treatments range	Control	Treatments range	Control
	percent			
Erambert	31-79	26	--	--
Stuart	37-54	39	--	--
W. Ouachita	40-52[1/]	35[1/]	12-34	11
E. Ouachita	26-64[1/]	31[1/]	7-33	12

[1/] 9 to 14 cm

Francis Marion - Piedmont loblolly pine

The orchard was fertilized in 1971, 1972, and 1973. Cones were collected in 1972, 1973, 1974, and 1975. Mean number of cones per tree were: 1972 - 1.0; 1973 - 9.8; 1974 - 15.6; 1975 - 5.9. Number of clones producing more than 10 cones per tree by years were: 1972 - 1; 1973 - 18; 1974 - 20; and 1975 - 6. Treatment affects on cone production were significant at the 5% level only in 1974. The Duncan range test showed that treatments with N and/or P_2O_5 levels of 224 to 364 kg/ha were significantly more effective than treatments with levels of 84 kg/ha or lower. Mean cone production per tree for N and P_2O_5 at rates of 0, 84, 224 and 364 kg/ha were: 12, 13, 18, and 24 for N and 11, 11, 18 and 18 for P_2O_5, respectively. Potassium fertilization did not affect cone production. The cyclic affect of cone production was evident in 1975 when mean yield per tree decreased to levels lower than those in 1974 and 1973.

Beech Creek - Cherokee shortleaf pine

Plots were fertilized in the Spring of 1971, 1972, and 1973, and cones

were collected in the Fall of 1972, 1973, and 1974. D.B.H. measurements were obtained for all trees; and grouped into three size classes: ≤ 9 cm; 9-14 cm, and > 14 cm. Analysis of the data for the 1974 cone crop showed that clones, tree size and treatments had significant effects on yield. Total cone production increased with time with yields of 690, 4941, and 9219 cones for 1972, 1973, and 1974 respectively. In 1974 one clone produced 27% of the total yield; 3 clones accounted for 54% of the yield; and 11 clones produced 84% of the yield.

Yield by tree sizes were highly significant, with mean yields per tree for all clones of 2.2, 14.7, and 25.8 for size classes 1, 2, and 3 respectively. Yields from the best clones were 13.9, 40.4, and 96.6 for size classes 1, 2, and 3 respectively. There was a direct relationship between cone production and rates of N. High rates (448 kg/ha) of P_2O_5 and K_2O had an adverse effect on production (Table 2).

Table 2.--Mean yield of cones per tree for different rates of nitrogen, phosphorus and potassium (Cherokee shortleaf pine).

Rates kg/ha	Cones per tree		
	Nitrogen	P_2O_5	K_2O
0	3.3	5.3	5.7
84	9.8	10.2	12.2
224	10.2	--	--
364	17.7	17.1	14.9
448	--	6.5	13.2

Table 3.--Mean cone yields per tree from best and poorest treatments - Cherokee shortleaf pine - all sizes.

Best treatments					Poorest treatments				
Rates kg/ha			All	Best	Rates kg/ha			All	Best
N	P_2O_5	K_2O	Clones	Clones	N	P_2O_5	K_2O	Clones	Clones
364	364	84	28.2	120.0	control			2.7	10.3
364	84	364	18.5	64.8	0	224	224	5.5	21.1
84	364	364	18.3	71.5	84	364	84	6.3	28.6
364	364	364	17.0	56.2	224	448	224	6.3	25.6

The high and the low treatments were significantly different from one or more other treatments (Table 3). The cyclic effect of cone production did not develop during the three years of collection.

Stuart - Louisiana loblolly pine

Plots were fertilized in March or April of 1972, 1973, 1974, and 1975. Cones were collected in 1973, 1974, and 1975. Total cone production was fairly constant for the three years with yields of 16189, 12224, and 12511 cones for 1973, 1974, and 1975 respectively (clone number 6 excluded). Clones and tree size were the only factors correlated with cone production. Three clones produced 72.9 percent, 13 clones produced 95.7 percent, and 23 clones produced 98.9 percent of the yield in 1974, while 9 clones did not produce any cones.

Tree size had a highly significant effect on cone production. Fertilizer treatments had no significant effect on cone yields, yet the fertilized plots produced twice the yield of control plots each year (table 4).

Table 4.--Mean number of cones per tree for Louisiana loblolly pine.

	1974	1975
D.B.H. < 9 cm - 54% of trees		
Treated plots	0.95	1.54
Control	0.77	1.27
D.B.H. > 9 cm - 46% of trees		
Treated plots	30.1	28.6
Control	17.4	12.2

Erambert - Alabama loblolly pine

Treatments were made on one set of plots in March 1972, 1973, and 1974 (Replication 1). A second set of plots were fertilized in 1973 and 1974 (Replication 2). Cones were collected from all trees in 1974, 1975, and 1976; and from Replication 1 (1972 plots) in 1973.

Only the 1974, 1975, and 1976 crops of replication 1 and the 1975 and 1976 crops of replication 2 were affected by treatments. Although trees from the two sets of plots contained the same clones and were comparable in age, the reactions to fertilizer treatments were different. Differences in cone production due to clones and tree sizes were significant at the 1% level for both sets of plots for all years. Treatment effects were significant at the 1% level for the 1975 cone crop - Replication 1; and at the 5% level for the 1976 cone crop, replication 1 and the 1975 cone crop, replication 2 (table 5).

Effects of nitrogen were significant at the 1% level for 1974 and 1975 cone crops in replication 1 and the 1975 cone crop in replication 2. Other variables significant at the 1 or 5% level for one or more crops were treatment x tree size, P, K, N x P, P x size, and N^2.

For trees smaller than 9 cm dbh, N and P significantly effected cone production in replication 1. An application of 364 kg/ha of N produced the most

cones for three consecutive years. Phosphorus at rates of 364 to 448 kg/ha of P_2O_5 gave the highest response. The best K rates were from 84 to 224 kg/ha of K_2O. For trees larger than 9 cm dbh, N had a significant effect on cone production for both replications and years. The optimum rate was 224 kg/ha of N each year except in replication 1, the 364 kg/ha rate was best for the 1976 crop.

Phosphorus and K apparently are not as critical as N in stimulating cone production in the Erambert Orchard. The highest yields per year sometimes obtained with the higher rates of P and K fertilization were not always significantly different from yields for other rates but they indicate the relatively wide range of rates that can be used. Potential optimum rates of P_2O_5 and K_2O are within the range of 84 to 224 kg/ha.

Table 5.--Rates of P_2O_5 and K_2O associated with the highest yields of cones - Alabama loblolly pine.

Variable	Years				
	1974	1975	1976	1975	1976
	P_2O_5				
All clones	Replication 1			Replication 2	
D.B.H. < 9 cm	448	448	224	0	0
D.B.H. > 9 cm	448	224	84	0	0
	Replications 1 & 2 Combined				
Best clones					
D.B.H. < 9 cm	448	364	364	--	--
D.B.H. > 9 cm	448	224	84	--	--
	K_2O				
All clones					
D.B.H. < 9 cm	224	84	84	224	84
D.B.H. > 9 cm	224	224	448	448	448
	Replications 1 & 2 Combined				
Best clones					
D.B.H. < 9 cm	448	84	84	--	--
D.B.H. > 9 cm	224	224	224	--	--

In 1975, the year of maximum cone production, only 18 clones produced an average of more than 40 cones per tree. Nineteen clones averaged less than 10 cones per tree. Only 16 clones had produced cones consistently over a three year period. When yields from all trees and from best clones were compared for specific periods of time, mean maximum yields for four year (1973-1976) and two year (1975-1976) period were obtained at annual rates of 364 kg/ha of nitrogen and 224 kg/ha of P_2O_5 and K_2O.

Ouachita - West and East Ouachita shortleaf pine.

Three sets of blocks were established in West Ouachita shortleaf pine in the spring of 1972 and two sets of blocks in East Ouachita shortleaf pine in the spring of 1973. Treatments were continued through 1975. For reference purposes each set of blocks will be considered a replication. Cone collection began in 1973 and continued through 1976.

West Ouachita

Clones, tree size, treatment and years (cycle) significantly affected cone production.

The highest yields were from treatments that contained N and/or P_2O_5 at rates of 224 kg/ha or higher. Analysis of variance for nutrients indicated that P, NP, NK, N^2, P^2, K^2 and NPK effects on large trees were significant at the 1 or 5% levels. Effects on small trees were generally non-significant. There was a reduction of cone production the third year from the cyclic effect. However, size 3 trees (> 14 cm dbh) were less affected than trees in the 9 and 9-14 cm diameter classes.

In 1975, the year of highest yields, only two clones produced more than 100 cones per tree, 5 clones more than 50 cones, 15 clones more than 20 cones and 24 clones more than 11 cones per tree. Twelve clones were consistent cone producers over a three year period. The larger trees with high yields generally required lower rate of fertilization than other trees (Table 6).

Table 6.--Fertilization rates for maximum cone production of West Ouachita shortleaf pine - kg/ha.

Years and tree size (dbh)	Nitrogen	P_2O_5	K_2O
	All clones		
1974 - 9-14 cm	--	--	--
1975 - 9-14 cm	224	448	448
1975 - 9-14 cm	224	224	224
> 14 cm	364	448	448
	High-yield clones		
1974 - 9-14 cm	364	84	364
1975 - 9-14 cm	224	364	364
1976 - 9-14 cm	224	224	224
> 14 cm	364	84	84

The accumulative effects of annual fertilization probably accounts for higher yields with lower rates of fertilization.

East Ouachita

Treatment effects on East Ouachita shortleaf pine were more pronounced than on the West Ouachita trees. Nutrient rates and interactions were highly significant for trees above 9 cm dbh. There were no real significant effects on small trees. Best rates for two years are shown in Table 7.

Table 7.--Fertilizer rates for maximum production of cones from East Ouachita shortleaf pine - kg/ha.

Year and tree size (dbh)	Nitrogen	P_2O_5	K_2O
	All clones		
1975 - 9-14 cm	364	364	84
1976 - 9-14 cm	364	84	84
> 14 cm	364	84	84
	Best clones		
1975 - 9-14 cm	364	364	84
1976 - 9-14 cm	364	84	84
> 14 cm	364	364	84

In 1976, the treatments with the highest yields were significantly better than treatments with lowest yields (Table 8); but all are not significantly different for each other or some other treatments. Low yields were associated with 0 rates of N, P or K and with the highest rates of P and K. These treatments were significantly lower than all other treatments.

SUMMARY

1. Fertilization to stimulate and maintain a vegetative cover may not be sufficient to stimulate flower and subsequent cone production. Fertilization may stimulate tree growth without affecting flower production.

2. The variability among seed orchards precludes the use of a standard rate of fertilization for all orchards.

3. Species and provenance affect efficiency of nutrient use and fertilizer requirements.

4. Tree size influenced cone production. Trees less than 9 cm dbh were not heavy cone producers and cone production on these small trees was not affected by fertilization.

5. Non-cone producing trees did not respond to fertilization.

Table 8.--Yields for East Ouachita shortleaf pine - 1976.

Rates N	P_2O_5 kg/ha	K_2O	Mean number of cones per tree: All clones All Sizes	All clones 9-14 cm	All clones > 14 cm	Best clones All Sizes	Best clones 9-14 cm	Best clones > 14 cm
					Best treatments			
364	364	364	82.4	56.1	150.0	170.0	109.3	227.9
364	84	84	76.0	66.7	115.0	141.9	136.0	178.5
84	364	84	58.0	44.4	107.3	123.5	106.3	158.2
364	364	84	50.0	46.2	83.4	100.0	88.3	143.4
					Poorest treatments			
224	224	0	2.2	2.8	3.4	4.4	5.8	4.0
224	224	448	3.2	3.8	3.0	5.1	6.3	4.2
224	0	224	5.0	6.6	5.0	9.3	11.3	6.6
0	224	224	6.0	5.1	22.5	12.9	9.4	32.1
224	448	224	8.3	8.5	14.8	16.1	17.6	20.4

6. Trees with a high potential for cone production were significantly affected by fertilization.

7. There must be a reasonable balance between N-P-K for the stimulation of cone production.

8. Nitrogen is the most important element affecting flowering provided P and K are not limiting.

9. Phosphorus and potassium are essential for cone production. High rates of fertilization may be used to increase levels of available and fixed P and K to optimum levels; after which heavy rates have an adverse effect on cone production. Optimum rates of fertilization after the first or second year are within the range of 84 to 364 kg/ha of P_2O_5 and K_2O. Rates of 448 kg/ha of P_2O_5 and K_2O had an adverse effect on cone production.

10. Rates of fertilization up to 224 kg/ha of N, P_2O_5 and K_2O did not break the cyclic effect of cone production by loblolly and shortleaf pine.

11. By judicious fertilization, the seed orchard manager may be able to produce abundant cone crops in different parts of the orchard each year - thus concentrating harvest on 1/3 to 1/2 of the orchard.

LITERATURE CITED

Barnes, R. L. and G. W. Bengston. 1968. Effects of fertilization, irrigation and cover cropping on flowering and on nitrogen and soluble sugar composition of slash pine. Forest Sci. 14(2): 172-180.

Clutter, Jerome L. 1968. Design and analysis of forest fertilization experiments. In: Forest Fertilization - Theory and Practice. T.V.A. Muscle Shoals, Alabama, pp. 281-293.

Cochran, W. G. and Gertrude M. Cox. 1957. Experimental designs. 2nd ed. John Wiley & Sons, Inc. N. Y. 611 pp.

Fuentes, Juan M. 1969. The use of fertilizers to increase slash pine (Pinus elliottii) flower and seed production. Tech. Note No. 29. Southlands Experiment Forest, Bainbridge, Ga. 3pp.

Greene, James T. and John N. Taylor. 1974. Stimulation of flowering in loblolly, slash and shortleaf pines. In: Proc. of colloquium on "Seed yield from southern pine seed orchards." Edited by J. Kraus. Ga. Forest Research Council. Macon. pp. 61-72.

Long, Ernest M., J. P. van Buijtenen, and John F. Robinson. 1974. Cultural practices in southern seed orchards. In: Proc. of colloquium on "Seed yield from southern pine seed orchards." Edited by J. Kraus. Ga. Forest Research Council. Macon. pp. 73-85.

Pritchett W. L. 1967. Fertilizing slash pine seed orchards. Cooperative Forest Genetics Research Program - 9th Progress Report, pp. 15-19. School of Forestry, University of Fla, Gainesville.

Schmidtling, R. C. 1969. Influence of rootstock on flowering in shortleaf pine. In: Proceedings of the Tenth Southern Conference on Forest Tree Improvement." Sponsored Publication No. 30. S.F. T. I. C. Eastern Tree Seed Laboratory. Macon, Ga. pp. 229-230.

Schmidtling, R. C. 1971. Cultivating and fertilizing stimulate precocious flowering in loblolly pines. Silvae Genetica 20(5-6): 220-221.

Schmidtling, R. C. 1975. Influence of fertilizer timing and formulation on flowering of young loblolly pine grafts. Final Report FS-50-1401-1.38.

Schultz, Robert P. 1971. Stimulation of flower and seed production in a young slash pine orchard. Southeast. Forest Exp. Stn. USDA Forest Serv. Res. Pap. SE-91. 10pp.

Schultz, Robert P., Carol G. Wells, and George W. Bengtson. 1975. Soil and tree responses to intensive culture in a slash pine clonal orchard: 12th year results. Southeast. Forest Exp. Stn. USDA For. Serv. Res. Pap. SE-129. 8pp.

van Buijtenen, J. P. 1965. The effect of spacing, fertilization and cultivation on flowering and seed production in loblolly pine. In: Proc. of the "8th Southern Conference on Forest Tree Improvement." pp. 141-147.

Varnell, R. J. 1976. Cone and seed production in slash pine: Effects of tree dimensions and climatic factors. Southeast. Forest Exp. Stn. USDA For. Serv. Res. Pap. SE-145. 10pp.

SEED ORCHARD FERTILIZATION: OPTIMIZING TIME AND RATE OF AMMONIUM NITRATE APPLICATION FOR GRAFTED LOBLOLLY PINE (PINUS TAEDA L.)

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Abstract.--Ammonium nitrate has substantially increased female flower production in two successive years, even when applied in addition to a high level of complete fertilizer. The response was highly variable by clone. There were no significant differences between times of application throughout the summer, and cone survival and production of viable seed were not affected. Therefore, the increase in female flower production due to fertilizer should be reflected in an overall increase in seed yield.

Key words: Pinus taeda, seed production, ammonium nitrate, seed orchards, female cones.

INTRODUCTION

Fertilization with nitrogen generally promotes flowering in conifers (see review by Puritch, 1972) and appears to be more effective on loblolly pine than either potassium or phosphorus (Schmidtling 1974 and 1975, and Webster, 1974). Loblolly pine responds well to nitrogen in the form of ammonium nitrate, ammonium sulfate, or sodium or potassium nitrate (John Robinson, personal communication, Western Gulf Tree Improvement Cooperative, 1977; Schmidtling, 1975). All three types of fertilizer had a significant promotive effect on female flowering by loblolly pine, but did not differ significantly from one another (Schmidtling, 1975). Whether nitrogen in the form of nitrate, ammonium, a combination of both, or some other form is most effective on loblolly pine requires further experimentation. In Douglas fir, the nitrate form is more effective than ammonium in promoting flowering (Ebell, 1972).

Most past demonstrations of the effectiveness of summer nitrogen were carried out on seed orchards or stands that had received little or no prior fertilization (Puritch, 1972; Schmidtling, 1974). In addition, Schmidtling (1974) found that response to ammonium nitrate varied greatly with the time during the summer when it was applied.

Since increases in flowering due to ammonium nitrate fertilization can be spectacular (a 300% increase for grafted loblolly pine has been reported by Schmidtling, 1974), this fertilizer has been used operationally in seed orchards for several years. At present, ammonium nitrate is applied to Weyerhaeuser's loblolly orchards at a rate of 75-112 kg N/ha (67-100 lb N/A) sometime

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in July, in addition to complete fertilizer applied in the spring and/or fall. The summer nitrogen is always applied, while the need for complete fertilizer is determined by analysis of soil samples.

Therefore, since complete fertilization in seed orchards is now routine, further information is needed to answer the following questions:

1) Is supplemental nitrogen effective even when applied in addition to a high level of complete fertilizer?

2) What is the optimum rate of application?

3) What is the optimum time of application?

METHODS

Ammonium nitrate was applied in the first half of May, July, and August at a rate of 224 and 448 kg N/ha (200 and 400 lb/A) and split applications of either 112 or 224 kg N/ha (100 or 200 lb/A) were applied in both May and August. The first treatments began in the spring of 1975 and have been applied yearly since then. Each treatment, including a control receiving no fertilizer, was applied to 5 ramets each of 5 clones for a total of 25 trees per treatment. All treatments were applied to the 11 year old (in 1975) North Coastal Long Fiber Orchard in Washington, North Carolina, while the July treatment was omitted from the five year old (in 1975) Flatwoods Orchard at Aliceville, Alabama. Both orchards have received 561 kg/ha (500 lb/A) of 10-10-10 complete fertilizer in late November every year since 1974.

Complete flower counts were made on all study trees each spring. Counts were made by two persons in a lift bucket, and were recorded if both counts were within 10% of each other. The first counts, made in 1975 prior to any fertilizer application, did not show any significant difference in the number of female flowers between the treatment groups used in the experiment.

In the fall, all the mature cones were harvested by clone and treatment, and if 50 cones per clone and treatment were collected, they were sent to the Eastern Tree Seed Laboratory in Macon, GA, for SOSET analysis. Data was obtained by clone and treatment on percent filled seed, seed germination, and total number of seed produced.

Results were analyzed by analysis of variance of the raw data and after a $\sqrt{n+1}$ transformation as used by Schmidtling, 1975. Treatment means were compared using both Duncan's multiple range test and Dunnett's statistic (Steele and Torrie, 1960).

RESULTS

The overall response of female flower production to different rates and times of ammonium nitrate application is summarized in Table 1. In both seed orchards, there has been an overall positive response to fertilizer in both 1976 and 1977. Although all treatments increased female flowering in 1976, analysis of variance did not reveal any significant overall treatment effects,

Table 1. Effect of rate and time of NH_4HO_3 application on ♀ flower production. Response as percent of untreated control. The actual number of flowers per tree for the control is given in the center column.

	224 kg N/ha (200 lb N/acre) Application Date					448 kg N/ha (400 lb N/acre) Application Date			
	May	July	August	May-August	# ♀/tree Control	May	July	August	May-August
Aliceville 1976	138%	-	145%	145%	40	113%	-	123%	140%
Aliceville 1977	189%	-	180%	206%*	54	144%	-	165%	217%*
Washington 1976	151%	153%	122%	147%	259	139%	125%	131%	136%
Washington 1977	224%*	237%*	218%*	240%*	280	194%*	170%	209%*	189%*
Overall $\bar{X}$, %:	176%	-	166%	185%	158	148%	-	157%	171%

*Significantly greater than control at 5% level (Dunnett's statistic).

although the response of some individual clones was significant (Figure 1). Variation between clones was highly significant. In 1977, all clones showed a much greater response to ammonium nitrate, and although differences between clones were again highly significant, ammonium nitrate had a significant (at the 1% level) effect on female flower production in both orchards.

Response at both orchards to different times of treatment did not significantly differ from each other either in 1976 or 1977. However, only the response to May-August treatment was significantly greater than the control in the Flatwoods Orchard in 1977 (see Table 1). In the North Coastal Long Fiber Orchard several treatments, including the split May-August treatment, were significantly greater than the control at the 5% level. While the split May-August treatment has given the best overall results, we cannot as yet conclude that the split application is the best method of application.

Trees at both orchards receiving 224 kg N/ha (200 lb N/A) flowered better than those receiving 448 kg N/ha (400 lb N/A). Comparing the response at each date for both sites in both 1976 and 1977, the 224 kg N/ha (200 lb N/A) rate performed best 12 of 14 times ($\chi^2 = 7.14$, which is significant at the 1% level) so that the lower rate of application appears to be significantly better.

The effect of the treatments on seed production by cones harvested from Washington, N.C. in 1976 is shown in Table 2. Although the flowers that gave rise to these cones were initiated prior to the first application of fertilizer, they completed most of their development after the first fertilizer treatments were applied. As yet, there are no significant differences in cone survival (expressed as a percent of the 1975 flower count) between the treat-

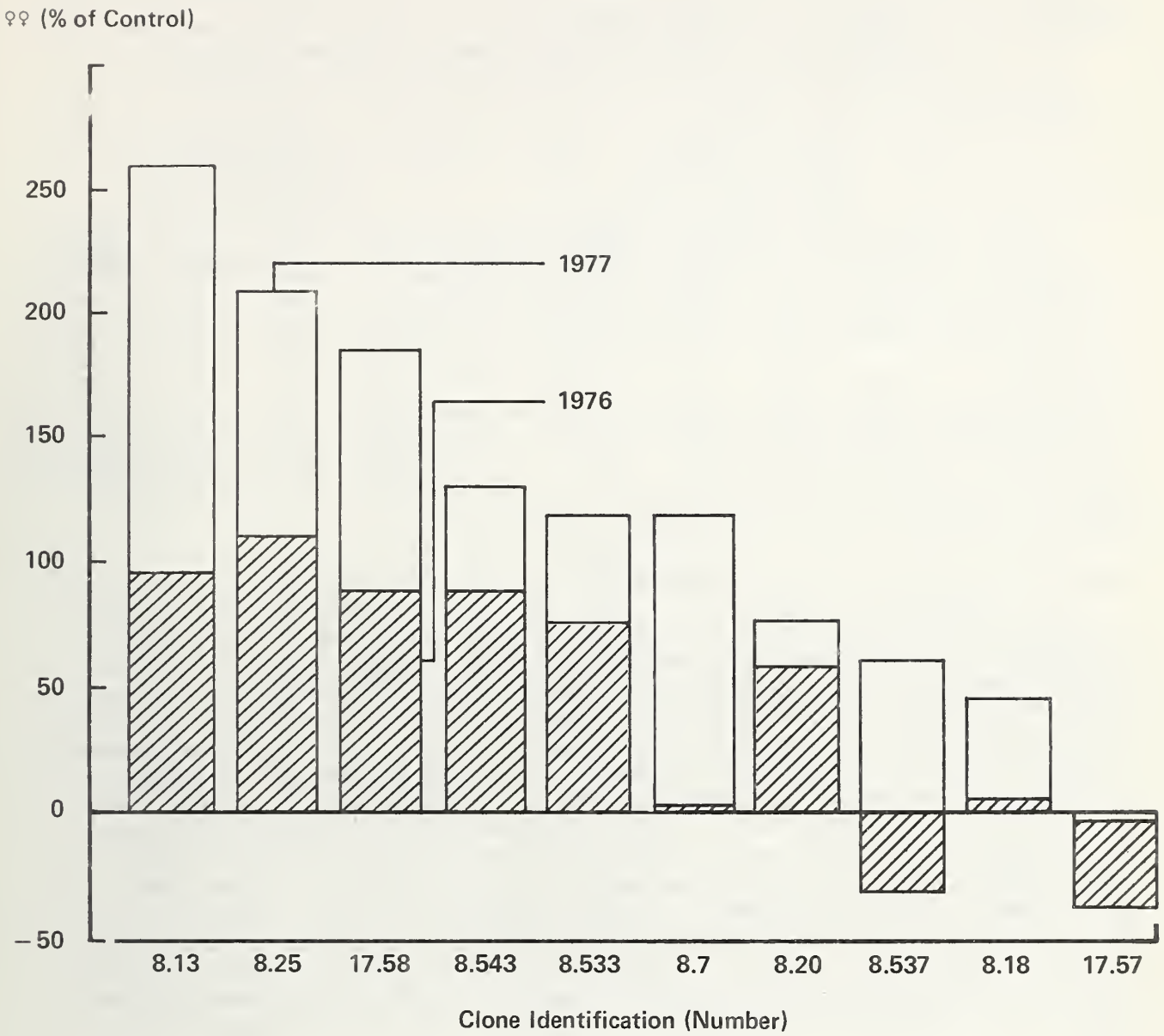

Figure 1.--Response of all 10 clones to split May-August treatment of 224 kg N/ha. The 0 line represents control response. The shaded portion of the bars represents 1976 response.

ments. In addition, there were no significant differences in percent filled seed, seed per cone, or percent germination between the treatments. Overall, fertilization does not appear to increase the yield of viable seed per cone.

Table 2. Effects of NH_4HO_3 fertilization on cone survival, percent filled seed, number seed per cone, and percent germination for the 1976 cone crop at Washington, North Carolina.

	224 kg N/ha (200 lb N/acre)					448 kg N/ha (400 lb N/acre)			
	Application Date					Application Date			
	May	July	August	May-August	Control	May	July	August	May-August
% ♀ Survival	31	39	48	49	47	52	54	47	44
% Filled Seed	81	73	64	74	73	74	69	81	81
# Seed/Cone	112	110	93	98	115	93	97	102	95
% Germination	95	93	93	93	96	89	92	95	90
Yield Index*	2672	2912	2657	3305	3788	3185	3325	3689	3047

*Number seedlings per 100 ♀ flowers.

CONCLUSIONS

Ammonium nitrate appears to stimulate female flowering in grafted loblolly pine, even when applied in addition to high levels of complete fertilizer, but has little effect on cone survival or viable seeds per cone. Therefore, the increase in flowering due to ammonium nitrate fertilization should result in an overall increase in seed yield. 224 kg N/ha (200 lb N/A) appears to be more effective at both orchard sites, with 448 kg N/ha (400 lb N/A) yielding a lesser response 12 of 14 times. Although Schmidtling (1975) found that August was the best time to apply ammonium nitrate, we have not as yet found a significant difference in treatment time, although the split May-August application was most frequently the best application. Schmidtling (1975) made a single application of ammonium nitrate at only 74 kg N/ha (66 lb N/A) to a previously unfertilized orchard, so his trees may have been much more sensitive to timing of application than the heavily fertilized trees of this study. In addition, much more ammonium nitrate was applied in this study, so a "carryover" effect may have occurred with the early application dates.

The lowest level of N (224 kg N/ha; 200 lb N/A) applied in this study was still at least twice the rate of operational fertilization. Therefore, more information is needed to establish the optimum rate of N application. However, volume growth response by loblolly pine to different rates of N fertilization varies greatly with soil type (Wells et al., 1976) and the same could be true for the flowering response at different orchard sites.

Despite the fact that most of the clones used in this study were chosen as uniformly good cone producers, there was still a great deal of variation by clone in female flower production and response to fertilizer. However, the response by clone in 1976 was highly correlated with that for 1977 ($r = .84$, significant at the 1% level, for correlation between clonal ranks for response to the 224 kg N/ha (200 lb N/A) May-August treatment in 1976 and 1977). In addition, the clonal response to fertilizer was directly correlated with intensity of flowering of unfertilized trees of the same clone ($r = .66$, which is significant at the 5% level, for rank of response to fertilizer vs rank of flowering intensity by the control trees).

Therefore, the relative response of the 10 clones used in this study was similar in both years, and the best flowering clones appear to also respond the best to fertilizer. "Customized" treatment by clone is possible, with only the best responding clones receiving fertilizer. Since these will also be the heaviest flower producers, the proportion of seed produced by these clones will be even higher with fertilization.

This study will be continued for at least two more years, so that we can continue to monitor the effects of heavy fertilization on flowering and seed production.

LITERATURE CITED

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

Puritch, G. S. 1972. Cone production in conifers. Information Report BC-X-65, Canadian Forestry Service, Pacific Forest Res. Centre, Victoria, B.C.

Schmidtling, R. C. 1974. Fruitfulness in conifers: nitrogen, carbohydrate, and genetic control. In Proc. of 3rd North American Forest Biol. Workshop, College of Forestry and Natural Resources, Colorado State Univ., Fort Collins, Colo., pp. 148-164.

Schmidtling, R. C. 1975. Fertilizer timing and formulation affect flowering in a loblolly pine seed orchard. In Proc. 13th Southern Forest Tree Improvement Conference, Raleigh, N.C., pp. 153-160.

Steele, R.G.D. and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., N.Y. 481 pp.

Webster, S. R. 1974. Nutrition of seed orchard pine in Virginia. Ph.D. thesis, N.C. State Univ. at Raleigh, Dept of Soil Science, 180 pp.

Wells, C. G., D. M. Crutchfield, and I. F. Trew. 1976. Five-year volume increment from nitrogen fertilization in thinned plantations of pole-size loblolly pine. For. Sci. 22, 85-90.

SESSION III - HARDWOODS

MODERATOR: GEORGE SWITZER

OPPORTUNITIES AND LIMITATIONS IN HARDWOOD TREE IMPROVEMENT RESEARCH AND DEVELOPMENT

Eyvind Thor 1/

Abstract.--Since there is such a great number of hardwood species, representing many genera and families, needs and procedures will vary greatly depending upon biological and economic factors and information already available. Prior to establishment of procedures it is essential to determine the importance of the species in question and how urgent it is to improve it.

Breeding programs with southern pines were initiated when forest managers determined an immediate need for increased production to supply their mills. Silvicultural practices, such as intensive site preparation, fertilization and increased stocking combined with shorter rotations would probably not result in sufficient production increases to account for the increasing demand for wood and the decreasing land base available for pine production. Under such circumstances, the possibility of increasing yield through breeding appeared very attractive even though gains from initial mass selection were expected to be modest. This urgent need for increased yields was the basis for a large number of "crash" breeding programs developed for southern pines. These programs, although generally successful, may be characterized as relatively expensive since development usually forged ahead of research and adjustments had to be made as research data became available.

When hardwood breeding programs were started a few years ago there was a strong temptation to copy procedures used in the successful pine programs. For many hardwoods there are, however, serious questions with regard to the urgency of the breeding programs. Since the degree of urgency may strongly influence breeding and research procedures it may be appropriate to evaluate each species as to the need for research and development programs. Such evaluations will, of course, to a large degree depend on the criteria used and the relative weight given each criterion (Farmer 1973). For the purpose of this paper it may be helpful to classify the native hardwoods into four "urgency groups":

1. Real urgency which is recognized
2. Real urgency which is not recognized
3. No real urgency but has been recognized as urgent.
4. No real urgency and has not been recognized as urgent.

Group 4 contains a large number of hardwood species, most of them of little or no commercial value. Since they are of no immediate concern to tree breeders they will be omitted from further discussion. In addition, there are some potentially important exotics; however, due to space limitations these hardwoods have also been omitted from this paper.

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"The author is grateful to King Features Syndicate Inc. for permission to reproduce Hagar comic strips in this paper."

REAL AND RECOGNIZED URGENCY

My friend and former country man, Hagar the Horrible, has agreed to illustrate a condition of real and recognized urgency (Figure 1). Some tree improvers can sympathize with Hagar in his predicament, but rather than waiting for some ladders they are looking for good research data to help them develop a breeding program.

Figure 1. -- The urgency is real and recognized.

Due to the large capital investment in the pulp and paper industry, needs related to wood procurement are readily recognized and seasonal shortages of suitable hardwoods have resulted in plantations of species such as cottonwood (Populus deltoides Bartr.), sweetgum (Liquidambar styraciflua L.), and sycamore (Platanus occidentalis L.). Improvement programs have been started with all three species.

Rapid growth rate and ease of vegetative regeneration promoted early selection work within the genus Populus. Our American cottonwood was introduced to Europe where it hybridized naturally with the European black poplar; selections of these hybrids were propagated vegetatively more than two hundred years ago. Artificial Euro-american hybrids created a number of different types which have entirely replaced native poplars in many countries. Such hybrids have not however, been successful in the Southern United States where selections of native cottonwood consistently outgrow all hybrids tested. In addition, native cottonwoods have better stem form and are more resistant to pests (Maisenhelder 1970). To take advantage of the high broad sense heritability in native cottonwood, selection in natural stands or plantations were followed by clonal tests. Such programs have resulted in the release of several clones for commercial plantations. The short rotations used in pulpwood plantations should permit relatively rapid clonal evaluation, particularly since there appears to be good genotypic correlations between measurements made in the third and sixth year (Mohn and Randall 1971).

In developing cottonwood clones with greater yields than those presently used, it will be necessary to maintain a large amount of genetic variation in the breeding population. Diallel crossing schemes can be used to develop new populations of known parental background. These populations may then be used for selection followed by clonal testing. Some parental types may be of exotic provenances; additional gains can be expected from the use of more southern material (Randall 1973) and inter-provenance crosses should be evaluated.

Although sweetgum and American sycamore are also recognized as belonging to the "real urgency" group, their brief silvicultural history is less glamorous than the saga recorded for cottonwood. There are two obvious reasons for this: Sycamore and sweetgum do not belong to genera with a large number of species and thus they are unlikely to produce valuable interspecific hybrids. Also, because they are much more difficult to propagate vegetatively it is not possible to utilize such a large proportion of the variation present. Research and development procedures reflect these differences.

Sufficient seed to provide for planting stock of the two species can easily be obtained from grafted orchards. Even though gains from phenotype selection in natural stands probably will be small, the cost of orchard establishment should also be modest due to the great number of seed produced per tree and the early age for flowering. However, to obtain significant genetic gains in these two species it is imperative to determine the variation among provenances and families within provenances.

The large geographic ranges of both species suggest that much seed source variation may be present. However, no range-wide studies have been established to determine variation patterns. Several studies, established by WGFTIP, the NC-Coop and the USFS Southern Forest Experiment Station, cover smaller parts of the southern range. The trees are still very young and offer only hints of geographic variation. Until more substantial information on provenance variation becomes available, breeders run the risk of selecting trees from inferior populations.

In most of the studies referred to above the identity of the open-pollinated progeny from individual mother trees has been maintained. In sweetgum the variation among families appears to be large (Wilcox 1970) indicating that selection based on progeny tests may result in significant gains. However, phenotype selection in natural stands did not result in genetic gains (Cooper 1974). These observations suggest that little emphasis should be placed on initial phenotype selection and that greater efforts should be made to establish open-pollinated progeny tests with a large number of more or less random selections from a substantial part of the species range. Better methods of vegetative propagation must be developed to take advantage of superior genotypes identified by this process.

REAL BUT NOT RECOGNIZED URGENCY

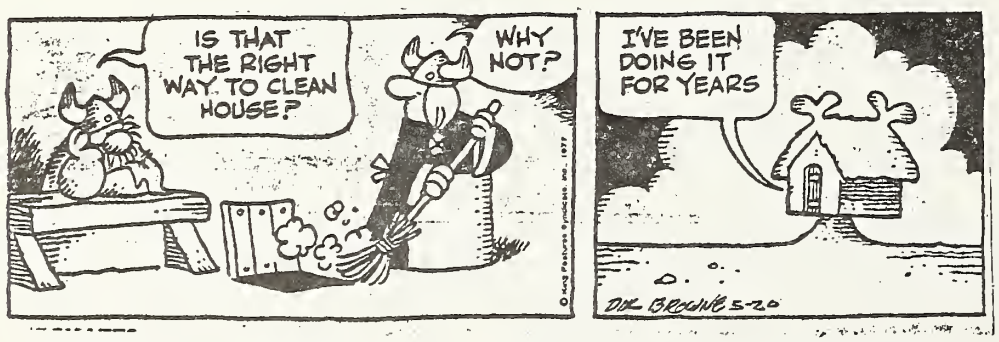

Figure 2. -- The urgency is real but is not recognized.

Hagar's wife illustrates a situation where there is a real urgency, but typically the matter has been ignored for years (Figure 2). Species belonging in this group will not be used by the pulp and paper industry at the present time and are not considered to be of great economic value. However, the overall national socio-economic values of these trees may be so high that the species deserve recognition by tree breeders.

Two species belonging to this group need special recognition: American chestnut (Castanea dentata (Marsh) Borkl.) and black locust (Robinia pseudoacacia L.). Chestnut is included because of its former great value as a timber and wildlife species and the fact that this genetic material is in danger of becoming extinct. Black locust deserves recognition since it is more widely planted than all other hardwoods.

Breeding work with the American chestnut was initiated by the US Forest Service, but when it became apparent that no quick solution to the problem could be anticipated the project was discontinued. As a result, it became a project of a few individual state organizations to solve what obviously is a national problem. The oldest projects are carried out at the Connecticut Agricultural Experiment Station (Jaynes 1976) and at The University of Tennessee (Samman and Thor 1976). Recently, breeding programs have been initiated at West Virginia University and Virginia Polytechnic Institute and State University. Several procedures have been followed since introduction of resistant exotics and interspecific hybridization failed to produce good forest trees. Attempts to produce resistant mutants by radiation are still being made, but so far with no success. Today, most of the work on chestnut blight (Endothia parasitica (Murr.) And. and And.) is concentrated in three fields: basic work on host-pathogen relationships, selection of apparently resistant phenotypes of American chestnut and progeny production, and development of a hypovirulent variety of E. parasitica.

There is considerable evidence that some chemicals in the inner bark of chestnut trees may either promote growth of the fungus or retard it (Samman and Barnett 1973). Work is in progress to determine the specific formula of these compounds. When determined, this information may be used to develop early selection criteria in progeny tests. Another project at The University of Tennessee attempts to determine the mechanism by which the fungus kills chestnut tissue; oxalic acid produced by the fungus is a prime suspect.

Although chestnuts continue to sprout from stumps, the number of live trees is declining. Such surviving trees are scattered miles apart through our eastern forests and since they require cross pollination there is no production of viable seed. To preserve this germ plasm and make it possible to produce new and possibly better recombinations, a program with phenotype selection (criteria: to be alive and greater than 10 inches DBH) and grafting in a clonal bank was initiated several years ago. This bank yields thousands of nuts used for establishment of half-sib progeny tests.

The difficulty in finding resistance within C. dentata has encouraged work with hypovirulence in E. parasitica The hypovirulent strain discovered in Europe has been mated with a virulent American strain of the to produce a hypovirulent American fungus which will, when introduced on cankered American chestnut trees, slow down the growth of the fungus and actually start

recovery (Van Alfen et al. 1975). If it becomes possible to establish this "sick strain" in our forests, trees with a relatively low degree of resistance to the virulent strains may be re-established.

Black locust does not generate nostalgia like the American chestnut; as a matter of a fact in some locations it is considered a weed species. However, the ability of this tree to fix nitrogen and become established on severely disturbed sites has made it a favorite among foresters engaged in strip mine reclamation. Thousands of acres of strip-mined land are planted annually with black locust making this species the most widely planted hardwood in the United States. Why then is there no recognized urgency for work with this species?

The answer is probably that people naturally tend to respond to economic needs that effect them directly. Strip mine operators usually do not have an interest in timber production and tree planting is only carried out to meet minimum requirements for release of compliance bonds and make the companies eligible for new mining permits. Timber production on such land is, however, of importance to the national economy and should be of public concern .

Foresters in Tennessee have observed that black locust planted on strip-mined land tends to grow with multiple stems and form brush thickets rather than forest stands. Even though site conditions may favor this development there is evidence that genetic factors may be important. It has been determined that the state nursery in Tennessee purchases black locust seed produced in western Europe. There is a good reason to believe that these stands originated from seed collected within the northern range of the species in New York. No attempt has been made to collect or test seed from native or other southern sources. The lack of information on genetic variation in the southern part of the species range indicates the need for a regional study of geographic and among-family variation.

Such a study, involving cooperation among the state forestry organizations in Kentucky and Tennessee, TVA, and The University of Tennessee, is now in the planning stage. Seed will be collected from at least a dozen natural stands in east Kentucky and Tennessee and north Alabama. Apparent clonal variation in growth form and borer resistance (Santamour 1970) indicates that some phenotype selection should be made. Test plantations will be established in strip-mined sites in Kentucky and Tennessee; in addition, a seedling seed orchard with half-sib families will be planted on a more conventional site. Following evaluation on the test sites for growth rate, stem form and resistance to insects, such as the locust borer and leaf miner, the orchard will be rogued by family and mass selection. Variation in rooting ability must also be evaluated. Since black locust flowers at an early age and early evaluation of many characteristics is possible,a clonal orchard can be established after about ten years. To take full advantage of the gains obtained it will be necessary to improve techniques for mass production of rooted cuttings (Stoutemyer et al. 1940). Since a relatively high plantation establishment cost is acceptable for successful reclamation of strip mines there are opportunities for development of new techniques such as production of containerized propagules.

NOT REAL BUT RECOGNIZED URGENCY

The third case of urgency, and the last one to be discussed, is the case when no real urgency exists but some people, due to hasty or maybe poor judgement, get carried away. In Figure 3 Hagar first deplores such rash actions, but then he gets involved in the general excitement and rushes off for what, at the moment, appears to be an important and urgent event.

Figure 3. -- There is no real urgency but it is recognized

There are some hardwood programs in the South which, I think, will fall into the third urgency group. To avoid straining some valuable friendships I will use as a prime example my own program with yellow-poplar (Liriodendron tulipifera L.). This program was started 17 years ago and has made some contributions to our knowledge of yellow-poplar (Thor 1975). The breeding program initiated was very similar to the crash programs developed for southern pines, but there was no similar economic justification for this approach. Even though a reasonable number of yellow-poplar seedlings were planted each year the demand for wood did not exceed production. As a matter of fact, Boyce and McClure (1975) concluded that "unless huge increases in demands occur, the present annual growth of 500 million cubic feet should be sufficient for many years to come." The rapid increase in net annual growth over the last 20 years has only been matched by a modest increase in removals resulting in a doubling of the growing stock volume. Such a situation should not call for urgent measures by tree breeders.

The lack of urgency due to large inventories of growing stock, high annual growth rates, modest harvests and long sawtimber rotations indicates that for yellow-poplar the emphasis should be on research in genetics rather than development of tree improvement programs. Such a research program, designed to determine the variation patterns within the species, has been developed by the S-23 Regional Technical Committee. This project, "Breeding strategies for genetic improvement of commercial forest trees in the South", includes basic studies in three hardwood species: yellow-poplar, sweetgum and American sycamore.

The S-23 yellow-poplar study may serve as an example for how forest genetics research can be carried out in a cooperative regional manner just as the three industry coops have served as models for developmental programs in tree improvement. The objective is to characterize genetic variation in natural populations and to determine optimal procedures for utilizing such variation in first-generation breeding strategies. Two provenance-progeny studies will be established, one based on regional collections and another based on altitudinal collections, to identify optimal sources and obtain narrow-sense heritability estimates by half-sib analysis of families within sources. Several cooperators will establish plantations so that by combined analysis it will be possible to determine family location interactions and stability of families evaluated. Based on this information we can determine probable success of different breeding methods.

Seed for the altitudinal study was collected at 1000-foot intervals from two transects across the Smokey Mountains. Seedlings will be distributed to cooperators in early 1978. Four transects (two N-S and two E-W) were used for regional collections; seedlings from two regional transects will be distributed in early 1978 while additional collections must be made for the two other regional transects. Tree breeders interested in participating in this program should contact Dr. Paul E. Barnett, Forestry, Fisheries and Wildlife, TVA, Norris, Tennessee 37828.

Another species which, in my opinion, qualifies for a "group three classification" is black walnut (Juglans nigra L.). The very high price paid for some individual trees and the propaganda by some hardwood manufacturers may suggest that an improvement program is urgently needed. However, our forests have been able to supply the industry with needed logs in addition to large volumes for export. Also, users of black walnut timber have not engaged in large planting programs to protect themselves against a future shortage. These facts indicate that an urgent need for an improvement program may not be present.

Breeding black walnut will in all probability require much effort. Trees are normally found as scattered individuals making phenotype selection in natural forests difficult and little progress should be expected from this approach. Selection in plantations is more promising, but can only be meaningful when good provenance test data are available. In many locations selection in plantations of known local source may be counter productive when trees of local source are inferior to those of exotic origin. Trees from as far as 200 miles south of plantations generally grow as large or larger than trees from local sources (Bey 1973).

Most provenance tests are designed primarily to show geographic variation patterns and are usually not well suited for carrying out individual tree selection. When a general geographic optimum area has been determined it is more efficient to make additional and much larger single-tree collections from this area to determine both ecotypic and among family variation. It is probably desirable to combine such progeny tests with seedling seed orchards (Funk 1969). However, roguing of inferior families and inferior individuals within the better families should probably not start before plantations are about 20 years old, when the trees are half way through the rotation and ready for heavy nut production. At this time it may be justified to establish clonal orchards from selections made in the progeny test plantation; this decision will to a large degree depend upon how strongly economically important characteristics are inherited,

if suitable methods of vegetative propagation have been developed, and if there is a strong demand for genetically improved seedlings. By including nut quality as a selection criterion the economics of grafted orchards may be greatly enhanced.

Several species of oaks (Quercus spp.) may be placed in the same urgency group as black walnut and the arguments for this classification as well as suggestions for breeding procedures will be similar. As a matter of fact, the large inventory of most oak species makes it even less urgent to develop clonal seed orchards. For large-seeded trees such as walnut and oak the cost per improved seedling tends to be excessive, particularly when vegetative propagation is difficult.

CONCLUSIONS

For a few hardwood species there is a real and urgent demand for improved seed. Phenotype selection in natural stands and establishment of clonal orchards are deemed appropriate for these hardwoods, especially when large amounts of seed can be obtained from relatively small orchards. Research aimed at the development of better methods of vegetative production, particularly mass production of rooted cuttings, should have high priority for these species.

For several other hardwoods of great economic importance there in no urgent need for improved seed. We will in most situations continue to rely on natural regeneration of these species. Until the economic conditions warrant large-scale plantings the development of clonal orchards should have low priority, especially for species yielding a relatively small number of seeds per tree. Research priority should be given to studies of variation within these species; future breeding efforts will depend on good estimates of both geographic variation and variation among families. These test plantations should include a large number of sources, families, and individual progenies so that they can be used for selection in future seed orchard development programs.

LITERATURE CITED

Bey, C. F. 1973. Growth of black walnut trees in eight midwestern States -- a provenance test. USDA For. Serv. Res. Pap. NC-91.

Boyce, S. G. and McClure, J. P. 1975. Capture of the biological potential for yellow-poplar timber. Proc. Third Annual Hardwood Symposium. p. 134-144.

Cooper, D. T. 1974. Performance of open-pollinated sweetgum progenies on contrasting sites in the Mississippi delta. Proc. of IURFO, Working Party on Progeny Testing S2-04.3. p.5.

Farmer, R. E. 1973. Decision making for development and use of genetically improved hardwoods. Jour. Forestry 71: 75-78.

Funk, T. 1969. Genetics of black walnut (Juglans nigra). USDA For. Serv. Res. Pap. WO-10.

Jaynes, R. A. 1976. New developments in chestnut research, Proc. 23rd Northwestern Forest Tree Impr. Conf. p. 36-41.

Maisenhelder, L. C. 1970. Eastern cottonwood selections outgrow hybrids on southern sites. Jour. Forestry 68: 300-301.

Mohn, C. A., and Randall, W. K. 1971. Inheritance and correlation of growth characters in Populus deltoides. Silvae Genetica 20: 182-184

Randall, W. K. 1973. Mississippi cottonwoods outperform local clones near Cairo, Illinois. USDA For. Serv. Res. Note SO-164.

Samman, S. N., and Barnett, P. E. 1973. Breeding for resistance to chestnut blight at The University of Tennessee. Proc. 23rd Northeastern Forest Tree Impr. Conf. p. 24-28.

Samman, S. and Thor, E. 1976. Breeding for resistance to chestnut blight at The University of Tennessee. Proc. 23rd Northeastern Forest Tree Impr. Conf. p. 24-28.

Santamour, F. S., Jr. 1970. Creating a seed orchard for superior black locust. Am. Hort. Mag. 49: 64-66.

Stoutemyer, V. T., Jester, J. R., and O'Rourke, F. L. 1940. Propagation of black locust clones by treating hardwood cuttings with growth substances. Jour. Forestry 38: 558-563.

Thor, E. 1975. Improvement of yellow-poplar planting stock. Proc. Third Annual Hardwood Symposium. p. 158-164.

Van Alfen, N. K., Jaynes, R. A., Anagnostakis, S. L., and Day, P. R. 1975. Chestnut blight: biological control by transmissible hypovirulence in Endothia parasitica. Science 189: 890-891.

Wilcox, R. 1970. Inherent variation in South Mississippi sweetgum. Silvae Genetica 19: 91-94.

ROOTING OF MATURE AMERICAN SYCAMORE CUTTINGS

G. Sam Foster and Eyvind Thor[1/]

Abstract.--Trees located on the western Highland Rim of Tennessee and varying in age from 13 to 31 years produced significantly different numbers of rooted stem cuttings. The percentage of rooted cuttings ranged from 83 to 8 percent, but was not related to age of ortet. Trees which produced greater numbers of rooted cuttings also had a significantly higher number of main roots and significantly longer main roots per cutting. In another experiment, using four mature sycamores growing in Knoxville, Tennessee, five different hormones were applied to girdled branch tips; only two (1 percent IAA and 0.8 percent IBA) surpassed the control in number of rooted cuttings.

Additional keywords: Platanus occidentalis L., cuttings, rooting, rooting hormones, variation in rooting.

American sycamore (Platanus occidentalis L.) has gained much attention by foresters in the United States due to its fast growth, favorable wood qualities for pulp and paper production, and large natural range. An ability to clonally propagate juvenile sycamore stem cuttings has been demonstrated by several authors (Briscoe, 1963; Hare, 1975; McAlpine, 1966; Nelson, 1957; Nelson and Martindale, 1957; Steinbeck and McAlpine, 1973).

Vegetative propagation of mature trees is important in tree improvement because superior selections are generally physiologically mature trees. Until recently, no method had been found to root mature material; however, Hare (1975) developed a technique for rooting sycamore cuttings which produced equal rooting success with stem cuttings from 6 and 13 year old ortets.

Knowledge of among-tree variation in rooting ability is needed in developing vegetative propagation procedures for clonal breeding work in sycamore. The inherent difference in rooting ability among sycamore trees has been alluded to by Hare (1975) but has not been directly tested.

One objective of this study was to evaluate natural variation in rooting ability of stem cuttings from mature sycamore trees using Hare's (1975) technique. The second objective was to compare six different rooting treatments.

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EXPERIMENT 1

Materials and Methods

Twelve trees ranging in age (at breast height) from 13 to 31 years (Table 1) were selected on upland sites on the western Highland Rim of middle Tennessee. The trees were selected in natural stands at a sufficient distance from each other to assure reproductive isolation, from 5 kilometers apart to approximately 80 kilometers apart.

Table 1.--Age, number of rooted cuttings per tree, average number of main roots per cutting, and average length of the longest main root per cutting obtained from the 12 selected upland sycamore trees.

Tree number	Age at breast ht. (years)	Number of rooted cuttings (24 attempted)	Average number of main roots per cutting	Average length of longest main root per cutting (cm)
I-18	17	20	21.7	5.2
I-23	17	14	18.2	5.1
I-5	16	14	11.5	3.8
I-3	20	13	15.3	3.4
I-1	31	12	16.1	4.9
I-2	13	12	7.3	2.5
I-9	13	11	17.0	5.7
I-21	14	8	3.3	2.3
I-7	20	5	3.6	1.7
I-22	27	3	8.2	1.6
I-10	13	2	5.0	0.6
I-13	21	2	1.5	0.3

On May 6-8, 1976, branch tips on each of the 12 trees were girdled, and girdles were covered with Hare's (1975) paste (1 percent indolebutyric acid; 1 percent 3-methyl-1-phenyl-5-pyrazolone; 20 percent sucrose; 5 percent Captan; 73 percent talc). Each branch tip (terminals and laterals were used) was girdled approximately 20 cm from the apical meristem using current year's shoots. The girdle was approximately 2 cm wide and did not completely circle the twig; a 1-2 mm bridge of bark, phloem, and cambium was left intact to help the twig survive until collection. A paste consisting of Hare's powder in distilled water was applied over the cut surface; the girdle was then wrapped with plastic wrap and aluminum foil tied in place by a copper wire. On each of the 12 trees, 30 twigs were girdled and treated although only 24 cuttings were needed for the experiment. The 6 extra cuttings allowed for some expected mortality. On June 1-2, 1976, the cuttings were taken from the trees and transported to Knoxville in buckets of ice water.

Greenhouse procedure.--The medium consisted of one part peat moss and one part perlite; heating cables provided bottom heat of 18-24°C. An inter-

mittent mist, of three seconds every three minutes, was applied from 8:00 a.m. to 6:00 p.m. A plastic tent was constructed around the mist benches to keep humidity high around the cuttings. Prior to placing the cuttings in the mist bench, the leaves were cut in half to reduce transpiration. Each cutting was dipped in Benomyl fungicide to prevent fungal growth and then in a powder containing 20 percent sucrose, 5 percent Captan, and 75 percent talc. The cuttings were spaced at 15.24 cm x 15.24 cm in the mist benches, and they were placed in a replicated design.

Cuttings remained in the mist benches for approximately one month. On June 28, 1976, the cuttings from the 12 selected upland trees were lifted and evaluated. Each cutting was evaluated for number of main roots per cutting, length of the longest main root, and whether callus was present.

Statistical analysis.--Because of the high number of non-rooted cuttings and therefore zero values, the frequency distributions were highly skewed to the low end of the scale; and consequently non-parametric tests were used for analysis. A Chi-square test was conducted for the relationship of number of rooted cuttings with ortets. This test was used because of the need to compare the distribution of rooted cuttings for a population of ortets with the expected frequency of equal rooting among cuttings from all ortets.

Kruskal-Wallis One-Way Analysis of Variance tests (Siegel, 1956) were used to analyze number of main roots per cutting and length of the longest main root per cutting. This test was used to analyze these two variables because each cutting which rooted has a value for both variables; and since there were unequal numbers of rooted cuttings among the ortets, the means would have to be used in a Chi-square test resulting in wasted information. The Kruskal-Wallis One-Way Analysis of Variance (Siegel, 1956) can be used when there are unequal numbers within each class and is a powerful non-parametric test.

Correlation coefficients were calculated for combinations of average number of main roots per cutting for each tree, length of longest main root per cutting for each tree, and percentage of rooted cuttings per clone.

Results and Discussion

The number of rooted cuttings was significantly different among ortets at the 1 percent probability level for the population of 12 upland trees. The observed values were the number of rooted cuttings per ortet; and since each ortet had an equal chance of rooting success, the population mean was the expected value. With 11 degrees of freedom, the Chi-square value was 36.58**. The range in rooting response among the 12 trees was quite large with the poorest tree producing 8 percent rooted cuttings and the best tree producing 83 percent rooted cuttings (Table 1).

Results of these tests indicate that phenotypes of sycamores differ in rooting ability and that some mature sycamore trees produce stem cuttings which have a high rooting percentage. That this strong among-tree variation

is not due to the age of the ortet is demonstrated for the upland trees by a non-significant correlation coefficient, r = -0.14.

To obtain an estimate of the quality of the root mass produced, the number of main roots and the length of the longest main root were recorded for each rooted cutting (mean values are in Table 1). The results of the Kruskal-Wallis One-Way Analysis of Variance tests (Siegel, 1956) revealed that the 12 upland clones are significantly different (1 percent probability level) in their number of main roots per rooted cutting and in the length of the longest main root per rooted cutting.

Correlation coefficients were calculated for the following combinations of three variables: average number of main roots per cutting for each tree x average length of longest main root per cutting for each tree, percentage of rooted cuttings per tree x average number of main roots per cutting for each tree, and percentage of rooted cuttings per tree x average length of the longest main root per cutting for each tree. The correlation coefficients were 0.92, 0.84, and 0.85, respectively, all significant at the 1 percent probability level. Although a cause and effect relationship cannot be drawn from correlation coefficients, it can be said that all three of these variables increase positively together. Apparently, cuttings from trees which have a better rooting ability also have more main roots and longer roots.

EXPERIMENT 2

Materials and Methods

On May 20-27, 1976, current year's shoots were girdled on four 27 year old trees selected on the Institute of Agriculture campus in Knoxville, Tennessee. Each branch tip (terminals and laterals were used) was girdled and the paste was applied as in Experiment 1. Ninety twigs were treated on each tree although only 72 cuttings were needed for the experiment. The 18 extra cuttings per tree were to compensate for any mortality taking place before harvesting of the twigs. Six treatments, consisting of a control and five growth regulators applied as a paste using distilled water, were used:

1. Hare's powder.
2. 0.8 percent IBA in 99.2 percent talc.
3. Control -- girdle only.
4. 1 percent IAA in 99 percent talc.
5. Hare's powder plus 10 ppm ethrel.
6. 0.8 percent IBA plus 10 ppm ethrel.

On June 30, 1976, the cuttings were taken and brought to the greenhouse.

Greenhouse procedure.--The greenhouse procedure in Experiment 2 was the same as in Experiment 1 except for using six hormone treatments instead of one. The cuttings were also placed in a replicated design as were the cuttings in Experiment 1.

The cuttings remained in the mist benches for approximately one month. On August 3, 1976, the cuttings from the four trees in the second experiment were lifted and evaluated. Again, each cutting was evaluated for number of main roots per cutting, length of the longest main root, and whether callus was present.

Statistical analysis.--Non-parametric tests were again used due to the desirability of not having to make the assumptions necessary for parametric tests. Chi-square tests were used to test the relationship between number of rooted cuttings and ortets and also between hormone treatment and number of rooted cuttings.

Friedman Two-Way Analysis of Variance tests (Siegel, 1956) were used to analyze the number of main roots per cutting and the length of the longest main root per cutting. Data were organized by clone and hormone treatment; clone-hormone treatment interactions cannot be determined with this test. This test was used because the data could be ordered in a two way table - by ramets and hormone treatments. In this case, the means were used because of the unbalanced nature of the data.

Results and Discussion

To date, only one technique has been presented in the literature (Hare, 1975) for rooting mature stem cuttings of sycamore. The experiment which compared Hare's technique with a check and four other chemical treatments revealed that while the reported technique gave some rooted cuttings, the check as well as the other four treatments produced a larger percentage of rooted cuttings. A Chi-square test revealed a significant relationship (at the 5 percent probability level) between hormone treatment and number of rooted cuttings (Table 2). The four ortet totals were pooled within each hormone treatment. The total number of rooted cuttings per treatment was the observed value, and the population mean was the expected value.

Table 2.--Effect of hormone treatments on rooting of cuttings from four sycamore trees and Chi-square test.

Hormone treatment	Number of rooted cuttings (48 attempted)	Percent of cuttings which rooted
1 percent IAA	30	63
0.8 percent IBA	21	44
Check	20	42
Hare's treatment + 10 ppm ethrel	16	33
0.8 percent IBA + 10 ppm ethrel	13	27
Hare's treatment	10	21

NOTE: DF = 5. $X^2 = 13.62$*.

*Significant at 5 percent probability level.

All the branch tips had calloused next to the girdled area and some had root primordia present. Only two of the hormone treatments exceeded the check treatment in the number of rooted cuttings produced. These two treatments utilized 1 percent IAA and 0.8 percent IBA and produced 63 percent and 44 percent rooted cuttings, respectively.

The number of rooted cuttings was related (significant at the 1 percent probability level) to ortets as shown by a Chi-square value of 22.16**. The six treatment totals were pooled within each ortet. The total number of rooted cuttings per ortet was the observed value, and the population mean was the expected value. Rooting ranged from 17 to 63 percent for the four clones.

As a further attempt to check the quality of the rooted cuttings, a Friedman Two-Way Analysis of Variance test (Siegel, 1956) was conducted on both the number of main roots per cutting and the length of the longest main root per cutting. No evidence was found that the hormone treatments differentially affected the two characteristics.

One major problem encountered with all clones and all treatments was leaf drop during the period cuttings were in the mist bench. Cuttings from some ortets tended to drop more of their leaves, but all the ortets had some leaf drop on their cuttings. In several instances, a cutting would root while the leaves were still alive; but when the leaves dropped, the cutting died within one or two months.

LITERATURE CITED

Briscoe, C. B. 1963. Rooting cuttings of cottonwood, willow, and sycamore. J. For. 61:51-53.

Hare, R. C. 1975. Girdling and chemicals promote rapid rooting of sycamore cuttings. USDA Forest Serv. Res. Note SO-202. 3 p.

McAlpine, R. G. 1966. Vegetative propagation methods for hardwoods. Proc. Eighth South. Conf. For. Tree Imp., p. 14-20.

Nelson, T. C. 1957. Rooting and air layering some southern hardwoods. Proc. Fourth South. Conf. For. Tree Imp., p. 51-54.

Nelson, T. C., and D. L. Martindale. 1957. Rooting American sycamore cuttings. J. For. 55:532.

Siegel, S. 1956. Nonparametric statistics for the behavioral sciences. McGraw-Hill Book Co., New York. 312 p.

Steinbeck, K., and R. G. McAlpine. 1973. Furrow planting American sycamore cuttings. USDA Forest Serv. Tree Plant Notes 24(4):22-24.

VEGETATIVE PROPAGATION OF MATURE SYCAMORE

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Abstract.--Techniques were tested for grafting, budding, and rooting cuttings from mature sycamore trees. Success was greater for winter and spring grafting (50-66%) and fall and winter budding (45-55%) than for rooting of hardwood cuttings (3%) or spring and summer budding (0-5%). Topophysis was present in grafts four years after grafting. Ortet ranks differed among propagation methods, and significant ortet variation for each method was not always associated with age. Waxed cleft grafts using scions from only the past year's growth increment are recommended for grafting sycamore, and fall T-budding can be used to extend the propagation period.

Additional keywords: Grafting, budding, rooting of cuttings, tree breeding, Platanus occidentalis.

Libby (1974) summarizes uses of vegetative propagation in tree breeding programs. The most common and probably most important present use is the movement of genotypes intact from scattered selected trees to a common site, usually called a clone bank or clonal seed orchard. Examination of vegetative propagation techniques for cloning selected trees in breeding programs of American sycamore (Platanus occidentalis L.) is the objective of the studies reported here. Ortet effects on success of various propagation methods are also evaluated.

Sycamore trees selected for a breeding program are usually physiologically mature. Little information is presently available on optimal grafting and budding techniques for such trees, and limb cuttings are hard to root. Kormanik and Brown (1974) and Hare (1976) have determined methods for increasing rooting success of cuttings from mature sycamore, but their methods have limited application for valuable, scattered trees in a breeding program. Foster's [2/] report is the first on ortet variation in rooting response for mature trees. Nothing has been reported on magnitudes of ortet variation and relative performances of ortets for other vegetative propagation techniques.

MATERIALS AND METHODS

In 1973, ten sycamore trees of ages 8, 11, 19, 21, 22, 22, 50, 55, 55, and 58 years at DBH from near Mississippi State University (33°14'N., 88°54'W.) were used as sources of vegetative propagules for four propagation studies. Treatments in the studies were selected based on results noted for other plants (Hartmann and Kester 1968).

Greenhouse Grafting Study

Side grafts were made on potted 1-0 rootstock brought into the greenhouse three weeks prior to grafting. All grafts were wrapped with a budding rubber and waxed with asphalt grafting compound. Treatments consisted of

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[2/] Paper presented in the current SFTIC Proceedings.

two collection-storage procedures for scions (collection and grafting without storage in mid-February vs. collection in mid-February followed by three-week cold storage before grafting) and two ages of wood at the "heel" of the scion (preceeding year's wood vs. two-year-old wood). A split-plot design with collection-storage procedures as whole units, four replications, and ten grafts per plot was used. Ortets were represented four times in each treatment combination. Success, as measured by survival, was recorded in June and September after grafting. Analyses of variance and Duncan's New Multiple Range Test of ranked means were used to test treatment and ortet effects on success.

Nursery Grafting Study

Scions were collected in mid February and placed in cold storage for four weeks before grafting on 1-0 nursery rootstock. All grafts were wrapped with budding rubbers. Treatments were two ages of wood at the "heel" of the scion (same as for the greenhouse study), two types of grafts (side and cleft graft), and three methods of protection of the new grafts from moisture loss (enclosing unwaxed graft and top of rootstock in a polyethylene bag, then covering with a kraft bag; waxing graft with asphalt grafting compound, but not enclosing in a bag; and neither waxing nor enclosing the graft). A randomized complete block design with treatments in a 2 x 2 x 3 factorial, four replications, and five grafts per plot was used. Ortets were represented twice in each treatment combination. Grafting success was measured in June and September, 1973. Degree of topophysis, where the graft was growing like a limb, was recorded on a scale of one (no topophysis) to three (major topophysis) in May, 1977. Analyses of variance and Duncan's tests were used on both the success and topophysis data.

Budding Study

The T-budding technique was used. Treatments consisted of winter budding (February collection and budding on greenhouse potted rootstock), spring budding (February collection and placement of budsticks in cold storage for five weeks before budding on nursery rootstock), summer budding (collection and budding on three-month-old nursery rootstock in late June), and fall budding (collection and budding on six-month-old nursery rootstock in late September). There were four replications containing five budded plants each for each of the first three budding dates and four replications containing twenty buddings each (two from each ortet) in the fall budding trial. Success was recorded in September, 1973, for the first three budding dates and in April, 1974, for the fall budding. An analysis of variance and a Duncan's test were conducted on the fall budding data for ortet effects.

Greenhouse Rooting Study

Six-inch hardwood cuttings having a "heel" of two-year-old wood and "wounded" on two sides at the base with a knife were basally dipped in a growth regulator before being planted or stored. The first treatment factor, growth regulators, consisted of either (1) a five second dip in a solution of 0.5% indolebutyric acid (IBA) and 0.5% naphthaleneacetic acid (NAA), followed by a dip in 5% captan powder (a fungicide), (2) a dip in 0.8%

IBA powder followed by a dip in 5% captan powder, or (3) a dip in 5% captan powder only. At planting, the cuttings were stuck four inches deep in one-gallon pots containing either sand or a 1:1 peat:perlite mixture, which represented the second treatment factor. The pots were placed on a mist bench in a greenhouse, with one cutting per pot. Four collection-storage procedures for the cuttings, representing the third treatment factor, were collection and planting without cold storage in (1) mid February (2) mid March, and (3) late September, and (4) collection in mid February with four-week cold storage before planting. A split-plot design with collection-storage procedures as whole units, four replications, and five cuttings per plot was used. Ortets were represented twice in each treatment combination. Survival was recorded in June and October for February and March plantings and in April, 1974, for the September planting. All cuttings were examined in April for presence of roots. Analyses of variance and Duncan's tests were used for the survival data and for percent of cuttings with root formation.

Comparison of Ortet Performance for Different Methods

Analyses of variance for randomized complete block designs with two replications were used to test significance of ortet variation for each method and trait. Ranked ortet means for age, percent success of each of the methods, and topophysis code were compared by Spearman's coefficient of rank correlation.

RESULTS AND DISCUSSION

Six months after grafting there was little difference between greenhouse and nursery grafting success (Table 1). Graft survival dropped from

Table 1.--Percent grafting and budding success of scions and buds collected from mature sycamore trees

Dates (Mo. & Day) in 1973		Location	Percent Success[a/]		
Scions or Buds Collected	Grafted or Budded	of Rootstock	June 1973	September 1973	April 1974
Grafting:					
Feb 9-12	Feb 13-14	Greenhouse	86	60 A	-
Feb 19-20	Mar 12-13	Greenhouse	75	50 A	-
Feb 19-20	Mar 19-22	Nursery	63	53	-
Budding:					
Feb 9-12	Feb 13-14	Greenhouse	-	55	-
Feb 19-20	Mar 28	Nursery	-	0	-
Jun 21	Jun 21	Nursery	-	5	-
Sep 26	Sep 27	Nursery	-	-	45

[a/] Means followed by the same letter are not significantly different at the 5% probability level.

June to September, indicating that grafting success should not be assessed before the end of the growing season following grafting. The mortality of apparently successful grafts may result from increased summer water stress in grafts with poor unions. Shock of moving the potted grafts 15 miles in an open truck from the greenhouse to a shadehouse in late June may also have affected ultimate success of the greenhouse grafts.

Neither date of collection-storage nor age of the "heel" of the scion had a significant effect on greenhouse grafting success (Tables 1 and 2).

Table 2.--Effects of location and treatment on grafting success and degree of topophysis of sycamore grafts from mature trees[a/]

Location and Treatment	Percent Success Six Months After Grafting	Degree of Topophysis in 4-Year-Old Grafts
Greenhouse:		
"Heel" wood age of scion:		
Previous Year's Growth (1972)	64 A	-
2-Year-Old (1971)	53 A	-
Nursery:		
"Heel" wood age of scion:		
Previous Year's Growth (1972)	61 B	2.2 G
2-Year-Old Growth (1971)	45 C	2.2 G
Type of graft:		
Side Graft	54 D	2.4 H
Cleft Graft	52 D	2.0 I
Protection from moisture loss:		
Not waxed, not covered	44 E	1.9 J
Not waxed, covered with bag	51 EF	2.3 K
Waxed, not covered	64 F	2.4 K

[a/]Means for a particular treatment and trait, when followed by the same letter, are not significantly different at the 5% probability level.

However, success in the nursery was greatest if the scion consisted of the previous year's growth increment and the graft was waxed, but not covered (Table 2). Side and cleft grafts gave equal success. The discrepancy between the two locations of grafting for significance of "heel" wood age may reflect differences in moisture stress at time of graft union formation or differences in precision of the two experimental designs. It is recommended from these results that scions consisting of the previous year's growth be used in either greenhouse or nursery grafting of mature sycamore, and that the grafts be waxed, but not covered.

After four years, 85 percent of the surviving nursery grafts exhibited some degree of topophysis, where the graft was growing like a limb. Side grafts exhibited a significantly higher degree of this effect than cleft grafts (Table 2). Possibly, tardy removal of rootstock tops

above the side graft union (done one month after grafting) could influence this result, but the persistance of the effects over four years makes this unlikely. The fact that unwaxed, non-covered control grafts gave less topophysis effect than the waxed or covered grafts cannot be explained. The recommendation here is to use cleft grafts for sycamore, since cleft grafts give equally as good survival as side grafts, are easier to make, and result in less topophysis effect.

Budding

Winter budding in the greenhouse and fall budding in the nursery were equally as successful as spring grafting in the greenhouse or nursery, but spring and summer buddings were failures (Table 1). At no time did the bark "slip" easily on sycamore. This difficulty with bark slippage makes T-budding of sycamore as slow as cleft grafting and more tedious. However, fall budding can be used to extend the period during the year when select sycamore trees can be successfully propagated in clone banks or clonal orchards.

Rooting of Cuttings

Survival of cuttings after seven months in the greenhouse was only three percent, even though up to 25 percent of the cuttings had evidence of root formation (Table 3). Even more cuttings initially leafed out,

Table 3.--Root formation and survival of cuttings collected from mature sycamore trees and planted on a greenhouse mist bench

Dates (Mo. & Day) in 1973		Percent Survival			Cuttings
Cuttings Collected	Cuttings Planted	June 1973	October 1973	April 1973	Having Root Formation (%)[a/]
Feb 9-12	Feb 14	13	2.5	-	14 A
Feb 19-20	Mar 23	28	1.7	-	15 A
Mar 22-23	Mar 23	34	2.5	-	26 A
Sep 26-27	Sep 27	-	-	5.8	13 A
Overall mean		25	------ 3 -------		17

a/Means followed by the same letter are not significantly different at the 5% probability level.

probably from stored food reserves, but died. It appears that rooting success of sycamore cuttings should not be determined strictly by presence of roots or by early leafing out, but rather by survival at least six months after planting the cuttings.

Neither growth regulator nor soil medium affected the percent of cuttings with roots, and a significant effect of growth regulators on survival was not meaningful in actual amount (Table 4). More recent work with nine of these same ten ortets [3/], using a 0.5% IBA and 0.5% PPZ (1-phenyl-3-methyl-5-pyrazolone) in 50% ethanol plus 20% sucrose and 5%

3/A cooperative study between R. C. Hare of the U. S. Forest Service in Gulfport, Mississippi, and the author.

Table 4.--Effects of growth regulator and soil rooting medium on root formation and survival of sycamore cuttings collected from mature trees and planted on a greenhouse mist bench [a/]

Treatment	Percent Survival Seven Months After Planting	Cuttings Having Root Formation(%)
Growth Regulator:		
0.8% IBA Powder + Fungicide	5.0 A	23 D
Fungicide (control)	3.8 AB	15 D
0.5% IBA + 0.5% NAA + Fungicide	0.6 B	13 D
Soil Medium:		
Sand	2.9 C	16 E
Peat:perlite (1:1)	3.3 C	18 E

[a/]Means for a particular treatment and trait, when followed by the same letter, are not significantly different at the 5% probability level.

captan in talc, has given up to 57 percent of cuttings with root formation. It appears that hardwood limb cuttings detached from mature sycamore trees can be rooted, but that the treatments of the present study were not appropriate.

Ortet Effects

Variation among ortets was significant for percent of cuttings with root formation, fall budding success, grafting success, and degree of topophysis exhibited by four-year-old nursery grafts (Table 5). Crown

Table 5.--Effect of ortet on root formation of cuttings, fall budding success, greenhouse grafting success, nursery grafting success, and topophysis of nursery grafts from mature sycamore trees [a/]

Ortet No.	Age at D.B.H. (yrs.)	Cuttings with Root Formation	Fall Budding Success	Grafting Success Greenhouse (%)	Grafting Success Nursery (%)	Degree of Topophysis
07	58	.5 E	37 FG	63 HI	30 N	2.15 PQR
01	55	.9 DE	7 G	31 J	29 N	2.85 O
06	55	10 CDE	37 FG	69 HI	83 K	2.10 QR
05	50	12 CD	63 FG	69 HI	38 MN	1.90 R
03	22	11 CDE	50 FG	44 IJ	63 KLMN	2.50 OPQ
08	22	8 CDE	37 FG	63 HI	42 LMN	2.10 QR
09	21	37 A	85 F	56 HI	75 KLM	2.20 PQR
10	19	2 DE	50 FG	63 HI	25 N	1.15 S
04	11	15 BC	63 FG	75 H	71 KLM	1.75 R
02	8	34 AB	16 G	50 HIJ	80 KL	2.65 OP

[a/]Ortet means for the same trait, when followed by the same letter, are not significantly different at the 5% probability level. Tests and means were obtained from % data transformed to arcsin $\sqrt{\%}$ for root formation and budding success, but were derived from non-transformed data for the other three traits. Maximum and minimum ortet means are underlined.

position effect should not be a contributing factor, since the means are based on many propagules taken from throughout the crown, (48 cuttings, eight fall buddings, 16 greenhouse grafts, and 24 nursery grafts per ortet). Age of ortet was not always important, since only one significant correlation between ortet ranking for age and for other traits was obtained: a negative correlation ($r = -0.66$) between age and percent of cuttings with root formation. It therefore appears that other factors, such as tree vigor and genotype, are primary influences on the ortet variation noted.

Ortets differed in relative rank from one method of vegetative propagation to another (Table 5). The only significant rank correlation was a positive one ($r = 0.71$) between percent of cuttings with root formation and percent grafting success in the nursery. Apparently, different physiological and (or) anatomical factors influence success of the different propagation methods. Genetic and environmental control of each factor may be independent of such control for other factors, so that an ortet would not react relatively the same as another ortet to different methods. Therefore, for establishment of clone banks or clonal orchards the inability to vegetatively propagate a selected tree by one method does not exclude the possibility of propagation by another method. For example, ortet 02 buds poorly, but roots and grafts well, while ortet 10 buds well and does poorly in rooting or nursery grafting. On the other hand, ortet 09 does well for all methods, and ortet 01 does poorly in all methods.

SUMMARY AND CONCLUSIONS

Under the conditions of these studies propagation success with physiologically mature sycamore propagules was better for winter and spring grafting (50-66%) and fall and winter budding (45-55%) than for rooting cuttings on a greenhouse mist bench (3%) or spring and summer budding (0-5%). Nursery grafts were more successful when the scion was taken from the previous year's growth increment than when it had a "heel" of two-year-old wood (61% vs. 45%). Waxed nursery grafts exhibited higher success (64%) than unwaxed grafts covered with polyethylene and kraft bags (51%) or unwaxed and uncovered grafts (44%). Side grafts and cleft grafts were equally successful in the nursery. Topophysis, where the graft was growing like a limb, was apparent in four-year-old nursery grafts and was significantly greater for side grafts than for cleft grafts. In greenhouse rooting of cuttings, neither date of collection and storage, growth regulator treatment, or soil medium had any meaningful effect on rooting success. Ortet variation was significant for percent of cuttings with root formation, fall budding success, greenhouse grafting success, nursery grafting success, and degree of topophysis on nursery grafts. Only ortet ranking for percent of cuttings with roots was significantly correlated with age of ortet ($r = -0.66$). Ortet ranks were usually not correlated for different propagation methods.

Based on these results, recommendations for vegetative propagation of mature sycamore trees in establishment of clone banks or clonal orchards are as follows:

(1) Until suitable methods are developed for rooting hardwood limb cuttings, grafting and budding should be used.
(2) For winter and spring grafting in the greenhouse or nursery use waxed cleft grafts and scions taken from the previous year's growth increment.

(3) Use T-budding in the fall to extend the period during the year when successful vegetative propagation can be accomplished.
(4) If difficulties are experienced in one method of propagating a select tree, try alternative methods (such as greenhouse vs. nursery grafting or spring grafting vs. fall budding).

LITERATURE CITED

Hare, R. C. 1976. Girdling and applying chemicals promote rapid rooting of sycamore cuttings. U.S.D.A., Forest Service, So. For. Exp. Sta. Res. Note SO-202, 3 pp.

Hartmann, H. T. and D. E. Kester. 1968. Plant propagation principles and practices (2nd ed.). Prentice-Hall, Inc., Englewood Cliffs, New Jersey. 702 pp.

Kormanik, P. P., and C. L. Brown. 1974. Vegetative propagation of some selected hardwood forest species in the southeastern United States. New Zealand J. For. Sci. 4(2): 228-234.

Libby, W. J. 1974. The use of vegetative propagules in forest genetics and tree improvement. New Zealand J. For. Sci. 4(2): 440-447.

SWEETGUM VARIATION CHANGES WITH TIME

Robert B. Ferguson and D. T. Cooper[1/]

Abstract.--An 11-year-old progeny test of open-pollinated sweetgum (Liquidambar styraciflua L.) at two sites in Mississippi indicates that conclusions drawn from early results (ages 2 and 6) may not apply at later ages. Genotype-environment interactions became less important between ages 6 and 11. The choice of test sites greatly affects the usefulness of results.

Additional keywords: Forest tree improvement breeding, genotype-environment interactions, selection, planting site.

Commercial growers are starting to plant sweetgum (Liquidambar styraciflua L.), causing increased interest in genetic improvement. Most reports on genetic research on sweetgum have concerned juvenile growth characters (Wilcox 1970, Mohn and Schmitt 1973, Sprague and Weir 1973), juvenile cell and wood characters (Randel and Winstead 1976, Winstead 1972, Webb 1964) or phenological characters (Williams and McMillan 1971). Little has been reported on trees older than age 6.

This paper reports results through age 11 from an open-pollinated progeny test grown on two diverse sites in Mississippi. Two previous papers discussed results from this experiment (Wilcox 1970, Mohn and Schmitt 1973). However, over time, changes in genotype-environment interaction have provided new information of importance to tree breeders.

MATERIALS AND METHODS

Forty sweetgum trees were chosen from throughout southern Mississippi to represent a range of phenotypes and sites. Open-pollinated seeds were collected and grown in a non-replicated nursery in 1962. The seedlings were outplanted at each of two locations in February 1963, using 4-tree linear plots, 12 foot equidistant spacing, and a 5-replication randomized complete block design. One site was on the Harrison Experimental Forest (HEF) near Gulfport, Mississippi (30°35'N latitude, 89°05'W longitude). The other site was on the Delta Experimental Forest (DEF) near Greenville, Mississippi (33°25'N latitude, 90°95'W longitude), 200 miles north of Gulfport. The soil at the HEF site is a well drained, strongly acid, Orangeburg fine sandy loam with low natural fertility. The soil at the DEF site is a poorly drained, slightly acid, Sharkey clay soil with high clay content and high fertility.

Height was measured to the nearest 0.1 ft. at ages 2, 6, and 11. At ages 6 and 11 dbh was measured to the nearest 0.1 in. Plot means were computed and examined by analysis of variance (Table 1). Estimates of components of variance were computed from mean squares. Within-plot variances were computed from individual tree data on all plots.

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Table 1.--Form of analysis of variance

Source of Variation	df	Expected Mean Squares
	Within location	
Replication	r-1	
Family	f-1	$\frac{\sigma^2_w}{n} + \sigma^2_{rf} + r(\sigma^2_{fp} + \sigma^2_f)$
Replication x Family	(r-1)(f-1)	$\frac{\sigma^2_w}{n} + \sigma^2_{rf}$
Within Plot	rf(n-1)	$\frac{\sigma^2_w}{n}$
	Over location	
Planting location	p-1	
Replication/Location	p(r-1)	
Family	f-1	$\frac{\sigma^2_w}{n} + \sigma^2_{rf(p)} + r\sigma^2_{fp} + rp\sigma^2_f$
Family x Location	(f-1)(p-1)	$\frac{\sigma^2_w}{n} + \sigma^2_{rf(p)} + r\sigma^2_{fp}$
Rep x Family/Location	(r-1)(f-1)p	$\frac{\sigma^2_w}{n} + \sigma^2_{rf(p)}$
Within Plot	rfp(n-1)	$\frac{\sigma^2_w}{n}$

Where n = harmonic mean of number of trees per plot.

Families and sites were considered to be random. Parent trees were not chosen strictly at random and sites were purposely chosen to represent contrasting environments in order to magnify genotype-environment interaction, producing conservative estimates of genetic gain.

Narrow-sense individual-tree heritabilities (h^2) were estimated as follows:

$$h^2 = \frac{4\sigma^2_f}{\sigma^2_w + \sigma^2_{rf} + \sigma^2_{fp} + \sigma^2_f}$$

where components of variance are: σ^2_f = family component

σ^2_{rf} = replication by family interaction

σ^2_{fp} = family by planting location interaction

σ^2_w = within plot

Standard errors for estimates of the family component of variance were obtained using procedures modified from Comstock and Robinson (1951) and then used to compute standard errors for estimates of heritability as follows:

$$SEh^2 = \frac{4SE\sigma^2_f}{\sigma^2_w + \sigma^2_{rf} + \sigma^2_{fp} + \sigma^2_f} \quad \text{(Becker 1967)}$$

Expected genetic gains (EGG) were computed for a hypothetical controlled crossing scheme involving the best tree from each of the eight best families. Procedures were modified from Namkoong et al. (1966). Standardized selection differentials (i) from Becker (1967) were used with pooled components of variance to estimate the expected genetic gains as follows:

Within location:

$$EGG_F = i_F \left[\frac{(\sigma^2_{fp} + \sigma^2_f)}{\left(\frac{\sigma^2_w}{rn} + \frac{\sigma^2_{rf}}{r} + (\sigma^2_{fp} + \sigma^2_f) \right)^{\frac{1}{2}}} \right]$$

$$EGG_{I/F} = i_{I/F} \left[\frac{3(\sigma^2_{fp} + \sigma^2_f)}{(\sigma^2_w + \sigma^2_{rf})^{\frac{1}{2}}} \right]$$

Over Location:

$$EGG_F = i_F \left[\frac{\sigma^2_f}{\left(\frac{\sigma^2_w}{rnp} + \frac{\sigma^2_{rf}}{rp} + \frac{\sigma^2_{fp}}{p} + \sigma^2_f \right)^{\frac{1}{2}}} \right]$$

$$EGG_{I/F} = i_{I/F} \left[\frac{3\sigma^2_f}{(\sigma^2_w + \sigma^2_{rf} + \sigma^2_{fp})^{\frac{1}{2}}} \right]$$

$$\text{Total Gain} = EGG_F + EGG_{I/F}$$

Location means and combined means for height over years were subjected to polynomial regression using the methods outlined by Graybill (1961).

RESULTS AND DISCUSSION

Height means at age 11 (Table 2) seem to indicate that the locations have similar production potentials, with the HEF location ($\bar{x}$ = 25.9 ft.) having a slight advantage over the DEF location ($\bar{x}$ = 22.8 ft.). However, closer examination of growth trends shows that height growth was initially rapid at HEF and slowed down with time; the reverse was true at DEF (Fig. 1). Mean dbh at age 11 was 3.52 in. at HEF and 3.29 in. at DEF. Although early dbh data were not available, growth trends from ages 6 to 11 were the same for dbh as for height. During the same period, diameter growth at HEF averaged only 0.27 in. per year while DEF averaged 0.38 in. per year. Only part of this difference can be accounted for by the amount of growing space, since survival was 81 percent at DEF and 97 percent at HEF. If present growth trends continue, the trees at DEF will eventually be larger than those at HEF.

Table 2.--Means and expected genetic gains (as a percent of the mean) for sweetgum planted at the Harrison Experimental Forest (HEF) the Delta Experimental Forest (DEF) and combined locations.

Character Location	Mean	Method of Selection[1/] Family	 Individual Within Family	 Combined
		-------% Expected Genetic Gain-------		
2 Year Height (ft.)				
HEF	7.24	5.9	10.0	15.9
DEF	3.15	6.9	11.6	18.5
Over Locations	5.19	5.3	8.4	13.7
6 Year Height (ft.)				
HEF	17.45	5.7	8.5	14.2
DEF	11.53	2.7	4.0	4.7
Over Locations	14.49	1.2	1.4	2.6
11 Year Height (ft.)				
HEF	25.93	5.7	8.7	14.4
DEF	22.76	2.2	3.4	5.6
Over Locations	24.34	2.0	2.5	4.5
6 Year Dbh (in.)				
HEF	2.16	7.1	11.1	18.2
DEF	1.39	5.4	8.3	13.7
Over Locations	1.78	4.5	6.1	10.5
11 Year Dbh (in.)				
HEF	3.52	7.2	10.9	18.1
DEF	3.29	2.6	3.6	6.2
Over Locations	3.41	3.7	4.9	8.6

[1/] Selection of the best tree from each of the best eight out of forty families.

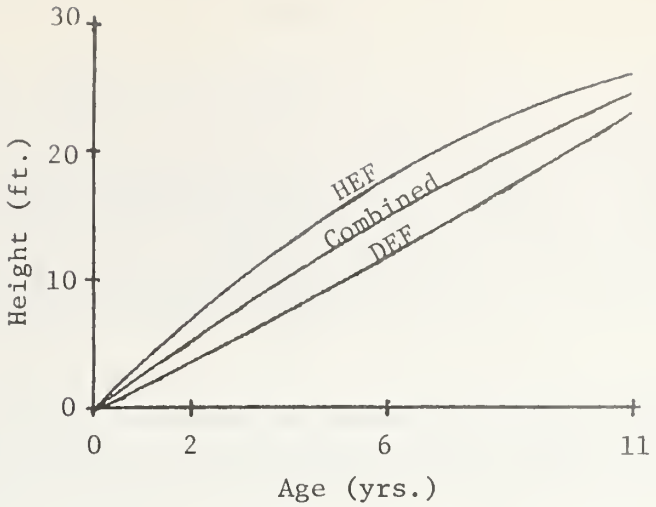

Figure 1.--The relationship of sweetgum height to age for the Harrison Experimental Forest (HEF), the Delta Experimental Forest (DEF), and combined locations

Differences in growth trends at the two sites were presumably due to soil characteristics. The well drained, acid, low fertility, fine sandy loam at HEF contrasts with the poorly drained, more nearly neutral, high fertility Sharkey clay at DEF. Despite these differences, both sites have similar problems which severely limit sweetgum growth. Good early growth at HEF probably occurred because the roots could penetrate the soil quickly. Later, as nutrient and moisture levels became limiting at HEF, growth rate decreased. Poor early growth at DEF was probably caused by the difficulty roots encountered in penetrating the soil, and the unavailability of moisture during the latter part of the growing season. As the roots finally penetrated the dense Sharkey clay at DEF, and were able to extract moisture from greater depths, growth rate surpassed that at HEF.

At age 2, the genotype-environment component of variance for height was less than half that of the genetic component of variance, but by age 6 it was five times as large. This trend reversed and by age 11 the genotype-environment component of variance for height dropped to less than twice the size of the genetic component of variance (Table 3). It remains to be seen whether genotype-environment interaction will continue to become less important or whether it will increase if the mean performance at DEF surpasses that at HEF.

Heritabilities were higher at HEF than at DEF (Table 3). They decreased through age 6 at both locations and then leveled off with the exception of dbh at the DEF location which dropped from 0.24 at age 6 to 0.16 at age 11. This decrease in heritability may have been a result of lower survival, giving extra growing space to many of the DEF trees and causing an increased non-genetic component in the denominator of the heritability formula. Heritabilities were rather low for data combined over locations because of high genotype-environment interactions.

Predicted gains based upon selection of the best eight of forty families were much lower at DEF than at HEF (Table 2) and were very low for data combined over locations. Predicted height gains for family selection over location were

5.3, 1.2, and 2.0 percent for ages 2, 6, and 11 respectively. Predicted gains for family selection for dbh at ages 6 and 11 were 4.5 and 3.7 percent. When gains for selecting the best individual within family were also considered, opportunities for gain were greatly increased. Predicted gains for combined selection for age 11 ht. were 14.4 percent at HEF and 5.6 at DEF. For age 11 dbh, predicted gains for combined selection were 18.1 percent at HEF and 6.2 percent at DEF.

Table 3.--Components of variance, heritabilities, and standard errors of heritabilites for the Harrison Experimental Forest (HEF), the Delta Experimental Forest (DEF) and combined locations.

Character	Variance Component				h^2	SEh^2
Location	σ^2_w	σ^2_{rf}	σ^2_f	σ^2_{fp}		
2 Year Ht						
HEF	1.3398	.1446	.1577	----	.384	±.067
DEF	.4128	.1440	.0519	----	.341	±.162
Combined	.9241	.1182	.0718	.0330	.250	±.106
6 Year Ht						
HEF	12.5231	.7666	.9694	----	.272	±.112
DEF	2.3160	.7917	.1543	----	.189	±.134
Combined	8.0320	.4780	.0994	.4610	.044	±.077
11 Year Ht						
HEF	26.9782	3.4861	2.2413	----	.274	±.121
DEF	5.0453	2.3834	.3975	----	.203	±.145
Combined	17.4618	2.1742	.4411	.8742	.084	±.021
6 Year Dbh						
HEF	.2991	.0543	.0256	----	.271	±.125
DEF	.0919	.0271	.0076	----	.240	±.139
Combined	.2070	.0349	.0086	.0080	.133	±.088
11 Year Dbh						
HEF	.8111	.0773	.0646	----	.271	±.116
DEF	.2419	.0581	.0124	----	.159	±.121
Combined	.5641	.0476	.0208	.0176	.128	±.081

Sweetgum does well on a wide range of sites, but this does not mean that a particular family or genotype will do well on an equally wide range of sites. Breeders need to know the amount of genotype-environment interaction present in their breeding populations. But the importance of genotype-environment interaction may change over time and may be influenced by the choice of sites. Genotype-environment interactions in this study were sufficiently high to suggest that much more progress can be made by breeding for a narrow range of environments than for a wide range of environments.

LITERATURE CITED

Becker, W.A. 1967. Manual of procedures in quantitative genetics. 2nd ed. Pullman: Wash. State Univ. Press. 130 p.

Comstock, R.E. and Robinson, H.F. 1951. Consistency of estimates of variance components. Biometrics 7: 75-82.

Graybill, F.A. 1961. An introduction to linear statistical models. Vol. 1. N.Y.: McGraw-Hill Book Co., Inc.

Mohn, Carl A. and Schmitt, Dan. 1973. Early development of open-pollinated sweetgum progenies. 12th South. For. Tree Improv. Conf. Proc. p. 228-232.

Namkoong, G., Snyder, E.B. and Stonecypher, R.W. 1966. Heritability and gain concepts for evaluating breeding systems such as seedling orchards. Silvae Genet. 15: 76-84.

Randel, William R. and Winstead, Joe E. 1976. Environmental influence on cell and wood characters of Liquidambar styraciflua L. Bot. Gaz. 137: 45-51.

Sprague, Jerry and Weir, R.J. 1973. Geographic variation of sweetgum. 12th South. For. Tree Improv. Conf. Proc. p. 169-180.

Webb, C.D. 1964. Natural variation in specific gravity, fiber length, and interlocked grain of sweetgum (Liquidambar styraciflua L.) in the South Atlantic states. Ph.D. Thesis. Raleigh: N.C. State Univ. Sch. of For. 138 p.

Wilcox, J.R. 1970. Inherent variation in south Mississippi sweetgum. Silvae Genet. 19: 91-94.

Williams, G.J. III and McMillan, C. 1971. Phenology of six United States provenances of Liquidambar styraciflua under controlled conditions. Am. J. Bot. 58: 24-31.

Winstead, J.E. 1972. Fiber tracheid length and wood specific gravity of seedlings as ecotypic characters in Liquidambar styraciflua L. Ecology 53: 165-172.

BLACK CHERRY SEED SOURCE STUDY IN NORTHERN ALABAMA - TEN YEAR RESULTS

Paul E. Barnett[1/]

Abstract.-- In 1965 seed of black cherry (Prunus serotina Ehrh.) was collected from 47 individual trees in eight widespread geographic areas--four in Tennessee and one each in North Carolina, Virginia, Pennsylvania, and Michigan. Outplantings were established at two widely separated locations in 1967. Performance at these locations is not explainable on the basis of source latitude and altitude alone, suggesting that other adaptive factors are involved. Height and dbh differences were highly significant after eight years in southwestern Michigan and after ten years in northern Alabama. Frequency of black knot infection was not related to source but differences among genetic families were significant.

Additional keywords: Prunus serotina, racial variation, geographic variation, provenance.

Black cherry (Prunus serotina Ehrh.) is a sufficiently important cabinet wood that the trees are sometimes valuable enough to be sold on a single-tree rather than a per-acre basis. It grows rapidly enough that planting and management are economically feasible land use alternatives (Wright and Lemmien, 1972).

The natural range of black cherry covers almost all of the United States east of the Great Plains and includes small populations in Mexico and Central America. However, the commercial range is limited to the Allegheny and Pocono Plateaus in Pennsylvania and adjacent areas in the Catskills and western New York. Although black cherry is not widespread in the southern Appalachians, high-quality trees are fairly frequent at higher elevations. Both quantity and quality of black cherry trees are much lower at the lower elevations of east Tennessee (lower than 2,000 feet) and on the Cumberland Plateau. This test was established to:

1. Determine the best source for black cherry seed suitable for planting at lower elevations in the Tennessee Valley.

2. Compare seedlings, and eventually trees, from various black cherry sources to see if either latitudinal or altitudinal races exist.

3. Observe morphological differences among sources and among trees within sources to determine the extent of the genetic variation for selected characteristics.

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This progress report presents a comparison of two provenance test plantations established in 1967 in southwestern Michigan and northern Alabama. The work of Jonathan W. Wright at the W. K. Kellogg Forest in Michigan is gratefully acknowledged.

MATERIALS

Seed was collected in 1965 from 47 individual trees located in eight widespread areas (Table 1). In most areas, an attempt was made to collect the seed from better-than-average trees located in a single timber stand. Since this was not always possible, only four of the Monroe County, Tennessee, trees were in the same stand; the remaining four were scattered but were growing at about the same elevation. All eight Pennsylvania trees from which seed was selected were in different stands.

Table 1.--Seed weights, altitudes, and sources per provenance for eight black cherry seed sources.

No. trees	Place of origin state, county	Latitude (County Seat)	Altitude feet/meters	Seed Per oz./gram	1-0 Seedling Ht. ft./cm.
6	TN, Anderson	36.06°N	800/ 243	482/17	2.01/61
4	TN, Giles	35.12°N	900/ 274	482/17	2.43/74
6	TN, Franklin	35.10°N	1,900/ 579	494/18	1.87/57
8	TN, Monroe	35.31°N	3,800/1,158	260/ 9	2.48/76
6	VA, Wise	36.56°N	4,000/1,219	292/10	1.78/54
2	NC, Macon	35.11°N	3,800/1,158	283/10	2.32/71
7	MI, Cass	42.17°N	700/ 213	295/10	2.47/75
8	PA, Cambia & Elk	40.29°N 41.20°N	2,000/ 610	288/10	2.27/69

METHODS

Both Michigan and Alabama test plantations were established in the spring of 1967 with 1-0 stock grown from seed at Norris, Tennessee. The Michigan planting (four replications, 10-tree plots) was established by Jonathan Wright on the W. K. Kellogg Forest near Battle Creek, in southwestern Michigan. The Alabama planting (12 replications, 20-tree plots) was established by Kingsley A. Taft, Jr., near Florence, in northwestern Alabama. Three other plantings established were not sufficiently intact by age 10 to warrant measurement and are, therefore, not included herein.

Plantings were established in the "compact family block" design, in which offspring of all parents in the same stand are grouped together. Wright and Lemmien (1972) have described the seven-year results of work at the Michigan plantation (six years in the field). An additional two years of field data are included in this report.

RESULTS

Michigan

Major results from the Michigan planting are presented in Table 2. It should be noted that this represents eight years in the field, since 10-year data were not available. As a result, performance at the two locations will not be directly comparable, but relative performance can be noted at the two locations.

At planting and after the second year, trees from North Carolina seed were the tallest and Virginia trees were shortest (Wright and Lemmien, 1972). All altitudinal relationship was noted by Farmer and Barnett (1972), which indicated a trend of larger black cherry seed with increasing source altitude and probably as a result, larger seedlings. The larger seedling size of Michigan and Pennsylvania trees is explainable on the basis of latitude (Table 1). Poor nursery performance by Virginia sources is an exception to this general observation; however, as will be noted, Virginia trees performed poorly throughout the life of the plantings in both locations.

Michigan trees were taller than all other sources at the Michigan site. However, Monroe County, Tennessee, source trees were taller than all remaining sources (the Giles County, Tennessee, source was not included in this planting). There was no difference in height among trees of the remaining five sources.

Michigan trees were larger in dbh than all sources except the Monroe County, Tennessee, trees. Monroe County, Tennessee, trees were larger than Macon County, North Carolina, trees, but there was no difference between Monroe County trees and the remaining four sources.

Table 2.--Eight and ten year performance of black cherry grown in Michigan and Alabama from seed collected in varying latitudes and altitudes.

No. families (trees)		Seed Origin State County	8-10-year[a] stock characteristics Height (m)		DBH (cm)		Black Knot Infected (percent)
MI	AL		MI	AL	MI	AL	AL
6	5	TN, Anderson	4.4	6.5	4.2	5.6	0.8
0	2	TN, Giles	-	6.3	-	6.1	5.5
6	6	TN, Franklin	4.4	5.9	4.1	4.8	11.6
7	8	TN, Monroe	5.2	4.6	4.8	3.8	3.2
4	3	VA, Wise	4.3	3.5	4.2	2.5	2.4
1	1	NC, Macon	4.3	4.0	3.4	2.8	4.5
7	5	MI, Cass	5.6	5.8	5.8	5.3	6.9
2	3	PA, Cambia & Elk	4.2	4.9	3.9	4.3	9.9
		F Value	11.4*	16.54*	6.8*	13.22*	1.90 N.S.

a. Michigan trees grown for 8 years in field; Alabama trees, 10 years.
*Significant at the 1 percent level.

Alabama

Major 10-year results of the northern Alabama planting are found in Table 2.

This planting was to consist of twelve 20-tree plots, but not enough seedlings were available to establish every family in every plot. In addition, because of heavy mortality due to poor drainage and honeysuckle and other competition, additional families were eliminated from some plots. As a result, only three replications included all eight sources by age 10. Analyses upon which this report is based were made on data from those replications. Other analyses were performed, which included more replications and fewer families, but no differences were observed in relative mean rankings.

The plantation had been established on a spacing of four feet in rows and 10 feet between rows. After the ninth growing season, it was marked and thinned to provide space for growth. Trees were left on the basis of height, diameter, and lack of black knot [Dibotryon morbosum (Schw.) T. and S.] infection. Five-year height data were analyzed again using data from trees left after thinning, so that all analyses are based on the same trees.

At planting and at two years in the field, North Carolina and Monroe County, Tennessee, trees were larger than trees from the remaining six areas but not significantly so. By five years, however, the Giles County, Tennessee, trees were tallest, and trees from the Michigan source were second tallest. North Carolina and Virginia trees were smallest after five years in the field.

Trees from Anderson County, Tennessee, were among the shortest when planted and after two years in the field, were fourth tallest after five years, and the tallest after ten years. However, there was no difference among trees in Anderson County, Tennessee; Giles County, Tennessee; Franklin County, Tennessee; and Michigan. Trees from North Carolina and Monroe County, Tennessee, which were tallest as 1-0 seedlings and after two years in the field, were only slightly taller than Virginia trees at 10 years. Virginia trees were shortest. Significantly, all three shortest trees are from southern high elevation sources and from seed and stock planted on low elevation sites.

Performance in dbh was similar to height growth. The top four sources in order of best performance were Giles County, Tennessee; Anderson County, Tennessee; Franklin County, Tennessee; and Michigan. Again, Monroe County, Tennessee; North Carolina; and Virginia were smallest in diameter, respectively.

Black knot is a disease which occurs on cherry and plum trees in orchards and also attacks wild species. On black cherry, large cankerous swellings two or more feet long occur on the trunks of large trees, and where such lesions are scattered along the bole, the tree is worthless (Boyce, 1961).

A large number of the trees in the Alabama planting were infected when thinned. These trees were removed, but the number of infected trees was recorded and the percentage infection was analyzed after transforming to arc sin $\sqrt{\text{percentage}}$. Although the range of variation among sources was wide, there was no difference in infection incidence among sources. There was, however, a significant difference among families.

DISCUSSION

In their earlier report on the Michigan planting, Wright and Lemmien (1972) noted that Michigan trees were most adaptable, growing nearly as well on knolls as in low areas. Performance in northern Alabama confirms this adaptability, since there was no height or diameter difference between Michigan trees and their low elevation southern counterparts.

Conversely, in Michigan after eight years, trees from the high elevation Monroe County, Tennessee, sources, while smaller in height and diameter than the local Michigan trees, were larger than more northerly sources and other high elevation southern sources.

In general, as would be expected, trees from the southerly, low elevation sources did better in the southern planting (with the exception of Michigan trees), and high elevation southerly sources performed better in the northern planting. Some of the trees from the southern high elevation sources were taller in Michigan after eight years than in Alabama after 10 years (Table 2).

As Wright and Lemmien (1972) suggest selections for growth apparently can be made from provenances. No significant growth differences were noted among families within sources.

In contrast to the situation with height and dbh, resistance to black knot appears to lend itself to breeding on an individual tree basis, with no difference noted among widespread sources.

LITERATURE CITED

Boyce, John S. 1961. Forest Pathology. McGraw Hill Book Co., Inc., New York, 3rd Ed., 261. p.

Farmer, R. E., Jr., and Paul E. Barnett. 1972. Altitudinal variation in seed characteristics of black cherry in the southern Appalachians. For. Sci. 18:169-175.

Wright, Jonathan W. and Walter A. Lemmien. 1972. Seven-year results from black cherry and black walnut provenance tests in Michigan. Proc. 8th Central States For. Tree Improv. Conf. pp. 61-44.

CHARACTERIZATION OF A COMPLEX PECAN-WATER HICKORY POPULATION IN SOUTHERN LOUISIANA

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Abstract.--Techniques of multivariate analysis were used to study the pattern of variation in leaf traits among pecan, water hickory and intermediate types growing on a disturbed site near Baton Rouge. Parental types were well-differentiated and a range of intermediate or hybrid phenotypes were encountered. A multidimensional plotting technique (MDPLOT) was used to explore the possibility that the hybrid trees were a mixture of F_1's and backcrosses to pecan. Analyses showed that the population has many of the characteristics of a hybrid swarm, and viable seeds were collected from the hybrid trees. The hybrid has potential as a timber tree.

Additional keywords: Hybridization, backcrossing, hybrid swarm, multivariate analysis, Carya illinoensis, C. aquatica, C. x lecontei.

Bitter pecan, Carya x lecontei Little, is the natural hybrid between pecan, C. illinoensis (Wangenh.) Koch, and water hickory, C. aquatica (Michx. f.) Nutt. It is found in bottomlands throughout the lower Mississippi Valley where the parental species are sympatric. Mature, forest-grown hybrids over 100 feet tall with good timber form have been identified on a number of sites in southern Louisiana (Adams and Thielges 1974). Seedlings grown from seed collected from these open-pollinated trees were 60% taller than pecan seedlings in a progeny test at age 2 (Adams 1976).

The taxonomy, distribution and ecological aspects of pecan-water hickory-bitter pecan populations were studied by Rousseau (1976). Distinctive nut characteristics aided in the preliminary identification of mature parental and hybrid types, and a supplementary method of individual tree validation based on leaf and leaflet characteristics was developed (Rousseau and Thielges 1977).

During these initial studies, it became obvious that hybrid trees were much more common than had been reported earlier. Also, trees validated as hybrids were fertile and produced viable open-pollinated seed (Rousseau 1976) which represent F_2 or backcross seedling progenies. The viability of these hybrid progenies suggests the distinct possibility for development, in nature, of genetically complex populations or hybrid swarms of these Carya species.

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Such a population was encountered during a search for hybrid seed in the vicinity of Baton Rouge, Louisiana. This paper reports the results of an exploratory analysis of population structure.

MATERIAL AND METHODS

A Carya complex is located just south of the Baton Rouge campus of Louisiana State University. It is a 100 acre site on the primary flood plain of the Mississippi River, between a high ridge or terrace (elev. 42 ft.) on the northeast and low flats before the river levee (elev. 18 ft.) on the southwest. The topography of the site is a series of parallel flats, sloughs and low ridges. The azonal bottomland soils vary with this topography and range from silty clay loams of the Commerce series on higher ridges to heavy, very fine clay soils of the Sharkey series on low flats and sloughs. The parental species are generally distributed according to site differences with pecan occupying the ridges and water hickory the low, wet flats and sloughs. The hybrid types tend to occupy intermediate sites, higher, better-drained flats and low ridges.

The site has been subjected to disturbances. Urbanization has isolated this population. Before that, the land was subject to periodic flooding by the Mississippi River. Moisture relations have been altered by successive phases of levee construction. Other major disturbances included initial land clearing, cycles of cropping, and grazing and abandonment. Road construction further altered drainage patterns. More recently, selective timber cutting and oil exploration and production have caused minor disturbances.

Unlike previously studied areas where hybrid trees were of approximately the same age, this population is unevenaged. Tentative identification of 44 trees were made on the basis of seed morphology. Twenty pecan, 11 water hickory, and 13 intermediate types were located for sampling.

Branches were collected from the four cardinal aspects at mid-crown on each tree. Two compound leaves were sampled from each of the four branches. The number of leaflets per leaf, rachis length (to the nearest 1.0 mm) and rachis diameter (to the nearest 0.1 mm) were determined on each of the eight leaves per tree. Six leaflets per leaf were selected for determination of number of serrations per 2 cm of leaflet edge, and amount of undersurface pubescence (visually estimated on a scale of 0-3).

These five traits had been previously determined to discriminate among pecan, water hickory, and hybrid types (Rousseau and Thielges 1976). Analysis of variance revealed highly significant differences among parental and intermediate types for all five traits. Results of these analyses were used to assign relative weights to each of the traits in the construction of a hybrid index. Two-dimensional scatter diagrams were plotted by using various combinations of traits as the X- and Y- axes.

Additional multivariate analyses were obtained by employing the multidimensional plotting technique developed by Andrews (1972) and programmed in ASA FORTRAN IV for the UNIVAC 1108 computer by Nance, et al. (1975). This technique, referred to as MDPLOT, provides for graphic, two-dimensional plotting of multidimensional (multivariate) data. Unlike scatter diagrams which are limited to

two-dimensional (bivariate) data in their interpretation, the MDPLOT also provides information on means (centroids) and interpoint distances in multidimensional space.

RESULTS AND DISCUSSION

A graphic presentation of hybrid index scores illustrates the distribution of individual phenotypes in the population (Figure 1). Scores ranged from 0 to 30 with the extremes representing classic or archetypal water hickory and pecan. The index effectively separated the parental species. The hybrid type is differentiated from water hickory. One tree, initially identified as hickory on the basis of its seed, was shown to be a hybrid.

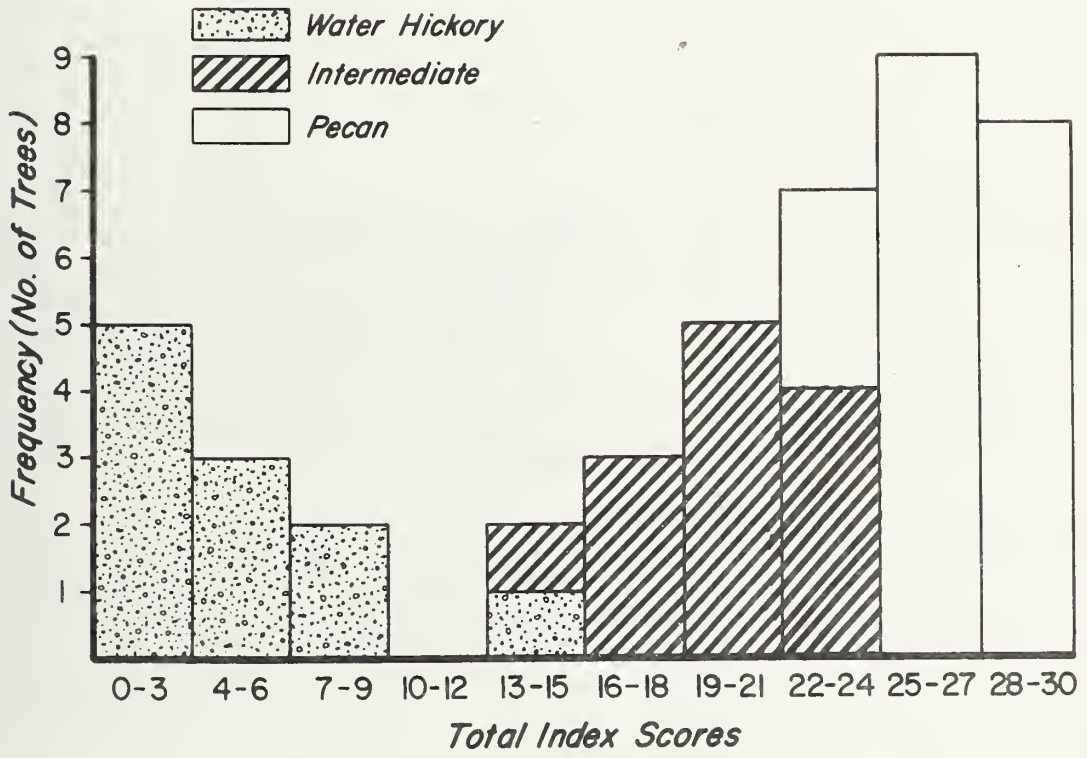


Figure 1.--Separation of parental and hybrid types in Carya complex on basis of hybrid index scores. Scores are based on (a) number of leaflets per leaf, (b) rachis length, (c) rachis diameter, (d) number of serrations per 2 cm of leaflet edge, (e) amount of undersurface pubescence, and (f) nut appearance.

There is a degree of overlap between pecan and the intermediate type and this pattern is again evident in the two-dimensional scatter diagrams (Figure 2). Parental types are well-separated and the misidentified water hickory plots squarely amidst the intermediate types.

The general relationships of Figure 2 were maintained when other combinations of traits were used as the X- and Y-axes in two-dimensional plotting. In viewing this spectrum of plots, a clustering of hybrid types was noted, eight of them (those between 6.2 and 7.3 serrations in Figure 2) tending toward intermediacy on all plots, while the other six were more generally scattered among the pecan parental types. This situation implies variable degrees of hybridity, specifically, F_1's and backcrosses to pecan.

To explore this possibility, the population was separated into four groups: pecan (20 trees), water hickory (10 trees), "F_1's" (8 trees), and "backcrosses" (6 trees). The latter two groups included those trees with hybrid seed type, but which varied from intermediacy to similarity to pecan in leaf and leaflet traits. Group means for all five variables were calculated from individual tree values, and the MDPLOT technique was applied to these data.

The computer-generated plots are illustrated in Figure 3. Major differences between plotting vectors for pecan (vector A) and water hickory (vector D) are evident in Figure 3A, and the hybrid vectors (B and C) are obviously intermediate. Separation of the "F_1" (vector C) and "backcross" (vector B) is also achieved at several points, notably at 0.06 on the X-axis.

From reference to tables of linear functions (Nance, et al. 1975), it was determined that the variables providing the strongest separation of the "F_1" and "backcross" vectors were number of serrations, degree of pubescence, and number of leaflets. The MDPLOT of these three variables provided better separation of the two hybrid vectors (Figure 3B). It must be emphasized that MDPLOTS of the 44 individual tree vectors do not result in such clear-cut separation of these four groupings. Pecan and water hickory are distinctly separated and hybrid types are, in general, intermediate. However, there is substantial overlap among individual trees of the two intermediate groups.

The techniques employed to this point do not provide positive validation of varying degrees of genetic intermediacy for individual trees, but they do supply evidence of backcrossing to pecan in this population that is discernible through multivariate analyses. Further evidence of backcrossing is provided by age and spatial distributions of intermediate types on the site and by the fecundity of these trees. Seeds were collected from 10 of the intermediate trees in this population and, while germination was only 20-35 percent as compared to 70-75 percent for pecan, all of them yielded viable progeny.

Some morphological and anatomical leaflet traits used in previous analyses (Rousseau 1976; Rousseau and Thielges 1977) were omitted from this study because they were found to contribute relatively little to differentiating among parental and hybrid types. However, it is possible that these additional variables would aid in discriminating between F_1 and backcross individuals. Nut characteristics such as weight, dimensions, color and texture may also be of value when included in multivariate analyses. These traits, as well as floral and phenological characteristics, will be included in further analyses. More positive information

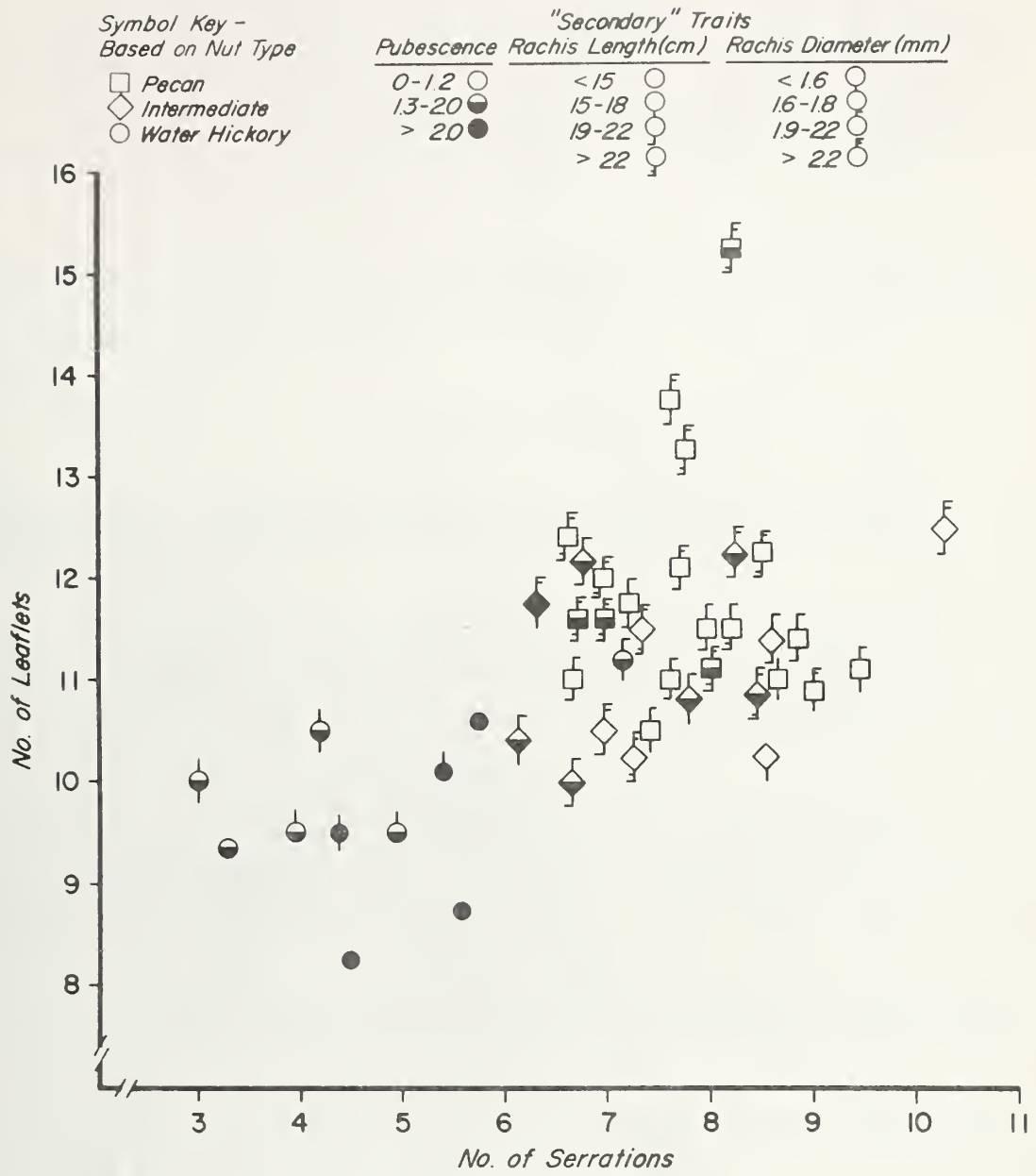
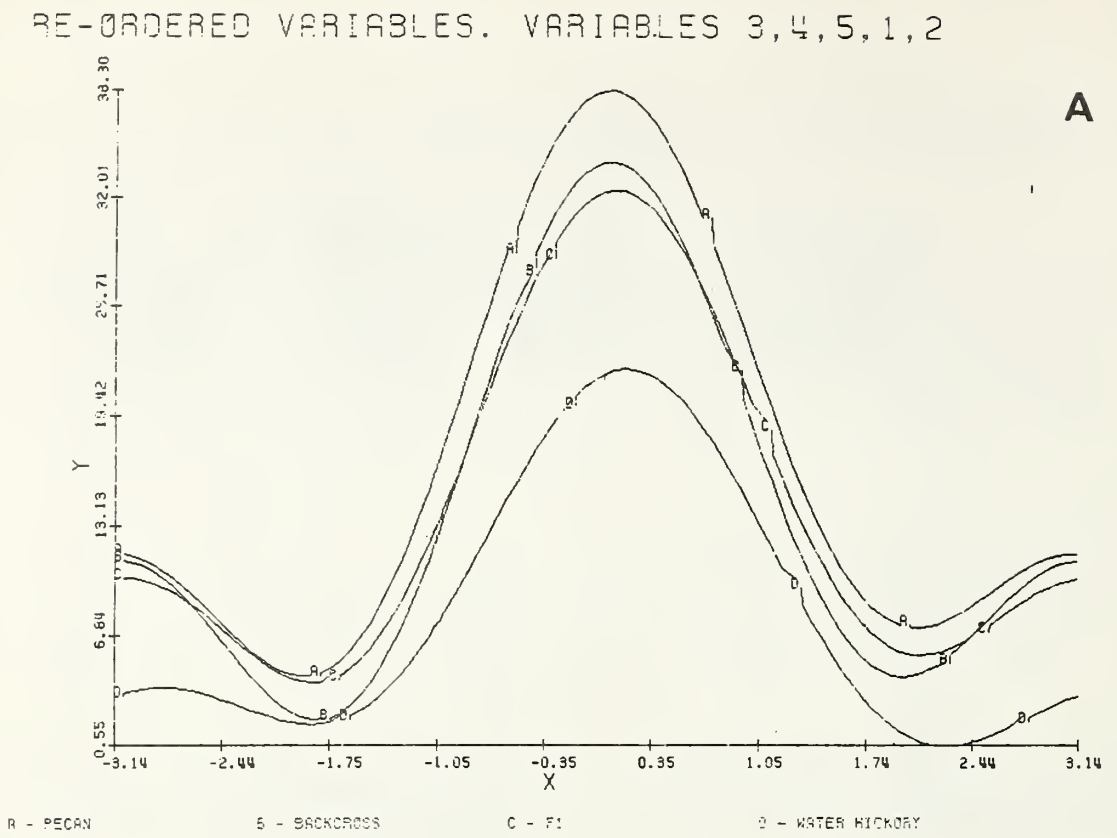


Figure 2.--Two-dimensional separation of parental and hybrid types in Carya complex on basis of leaf and leaflet traits.



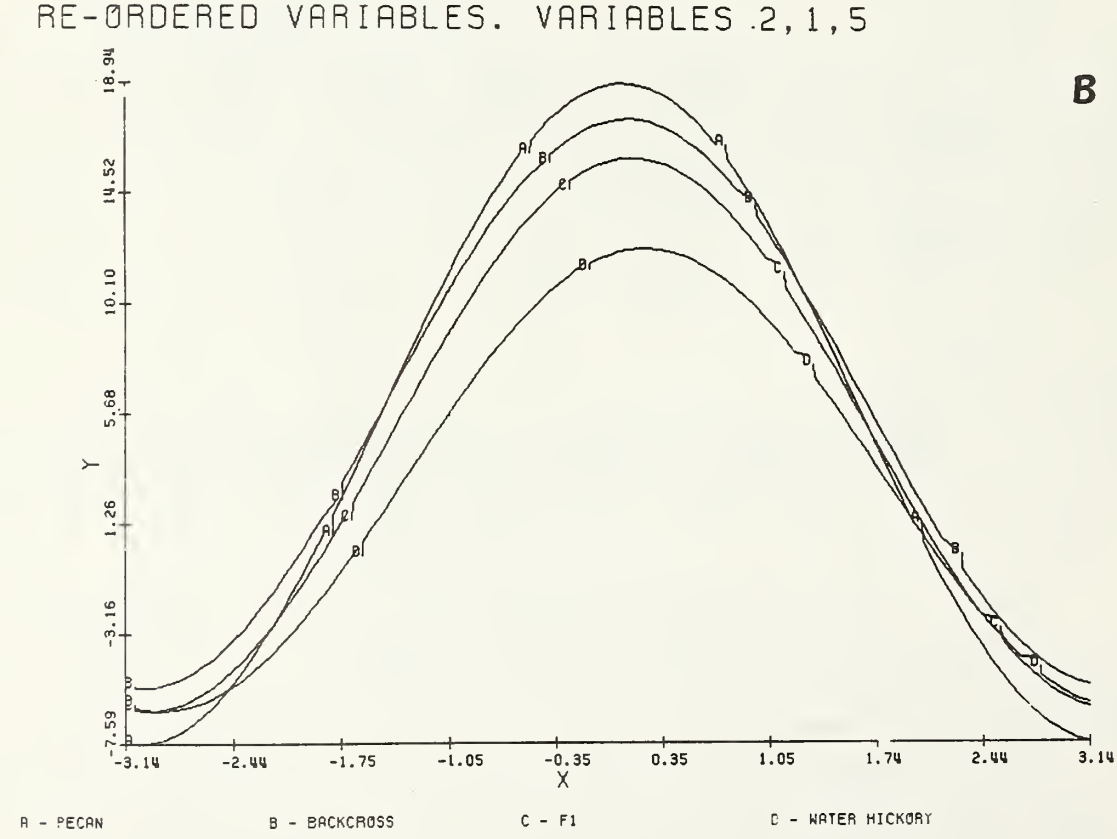


Figure 3.--Computer-generated MDPLOTs separating parental species and hybrid types in Carya complex. A - all five variables plotted. B - three variables plotted.

on degree of hybridity could be obtained through production of F_1, backcross and F_2 progenies through controlled pollination. These progenies would provide baseline data or standards for each trait which could be used to evaluate intermediate types in wild populations.

This large population of intermediate types suggests that isolating mechanisms between pecan and water hickory are of an environmental rather than genetic nature. Hybrid trees are fertile and produce viable progeny. Moreover, the tendency of hybrid individuals to occupy intermediate areas of this disturbed site closely fits the criteria for the classical hybrid swarm described by Anderson (1949). More intensive studies will aid in determining if there is introgression in this population.

From a more practical standpoint, hybrid progenies appear to have good potential for wood production. Further characterization of the genetic makeup of parent trees and observation of test progenies should provide useful information for selection and breeding programs.

LITERATURE CITED

Adams, J. C. 1976. A study of genetic variability in wild populations of pecan (Carya illinoensis (Wangenh.) Koch. Ph.D. Dissertation, Louisiana State Univ., Baton Rouge. 111 p.

Adams, J. C. and B. A. Thielges. 1974. Genetic improvement of pecan for wood production. Centr. States For. Tree Improve. Conf. Proc. 9: 159-163.

Anderson, E. 1949. Introgressive hybridization. Wiley and Sons, New York. 109 p.

Andrews, D. F. 1972. Plots of high-dimensional data. Biometrics 28: 125-136.

Nance, W. L., B. H. Polmer and G. C. Keith. 1975. MDPLOT: A program for plotting multi-dimensional data. USDA For. Serv., Gen. Tech. Rep. SO-7. 26 p.

Rousseau, R. J. 1976. A taxonomic and genetic study of Carya illinoensis, C. aquatica and their hybrid, C. x lecontei. M. S. Thesis, Louisiana State Univ., Baton Rouge. 83 p.

Rousseau, R. J. and B. A. Thielges. 1977. Analyses of natural populations of pecan, water hickory and their hybrid, bitter pecan. Centr. States For. Tree Improve. Conf. Proc. 10: 1-8.

TOLERANCE OF COTTONWOOD TO DAMAGE BY COTTONWOOD LEAF BEETLE

F. L. Oliveria and D. T. Cooper[1/]

Abstract.--Wide variation in tolerance to the cottonwood leaf beetle was found in fourteen hundred eastern cottonwood clones, originating from 36 young natural stands along the Mississippi River from Memphis, Tennessee, to Baton Rouge, Louisiana. Expected genetic gains were large enough to justify further research.

Additional keywords: Host resistance, defoliation, Populus deltoides, Chrysomela scripta.

The cottonwood leaf beetle (Chrysomela scripta F.) causes defoliation and damage to young terminals in eastern cottonwood (Populus deltoides Bartr.). Severe infestations result in growth loss and reduce the quality of the main stem by causing crooks and forks. Damage is particularly severe in plantations along the lower Mississippi River.

Cottonwood trees can be protected from the leaf beetle with chemical insecticides. However, the need for precise timing and repeated applications, high costs, loss of desirable predators, and probable development of insecticide-resistant strains of the leaf beetle point to the need for cottonwood clones genetically resistant to the leaf beetle.

European scientists have studied insect resistance in plantation poplars for several decades (Arru 1975). Benjamin and Berkot[2/], at the University of Wisconsin, are studying leaf beetle resistance of cottonwood clones developed from callus tissue. No resistant clones are available in the United States.

MATERIALS AND METHODS

During fall 1971, about 40 clones from each of 36 two- to four-year-old stands of cottonwood along the Mississippi River from Memphis, Tennessee, to Baton Rouge, Louisiana, were selected for study. Within each stand, trees were chosen essentially at random. The amount of natural selection within stands and the number of parents contributing to each stand were unknown. The trees were cloned and maintained in the nursery for 4 years before an outplanting was established in February 1976 on the Fitler Managed Forest, 50 miles south of Greenville, Mississippi.

The 36 stands were arranged as a 6 x 6 triple lattice with 40 clones randomized within each stand in each of the three replications. Plot size was two trees. Spacing was 12 x 12 feet. Three 20-inch cuttings were used at each planting spot and thinned to the one best tree in July. Thus, most clones were represented by three two-tree plots at the time the trees were scored.

1/ Associate Entomologist and Plant Geneticist, at the Southern Hardwoods Laboratory, which is maintained at Stoneville, Mississippi, by the Southern Forest Experiment Station, USDA Forest Service, in cooperation with the Mississippi Agricultural and Forestry Experiment Station and the Southern Hardwood Forest Research Group. The authors thank Crown Zellerbach Corporation for providing the planting site.

2/ Personnal correspondence with D. M. Benjamin and T. Berkot, University of Wisconsin-Madison, Department of Entomology, Madison, Wisconsin 53706.

The trees were scored for cottonwood leaf beetle damage during the last two weeks of October 1976. The amount of damage on the upper 24 inches of the main stem was ranked on a 1 to 11 scale as follows:

1 - leaf damage 0-10%, no terminal damage, active growth
2 - leaf damage 0-10%, no terminal damage, no active growth
3 - leaf damage 10-25%, no terminal damage, active growth
4 - leaf damage 10-25%, no terminal damage, no active growth
5 - leaf damage 25-50%, no terminal damage, active growth
6 - leaf damage 25-50%, no terminal damage, no active growth
7 - leaf damage 50-100%, no terminal damage, active growth
8 - leaf damage 50-100%, no terminal damage, no active growth
9 - leaf damage 50-100%, terminal damage
10 - leaf damage 25-50%, dead terminal
11 - leaf damage 50-100%, dead terminal

Thus, data did not represent a true interval scale and analysis of variance procedures are not strictly applicable. However, they were used since methods of estimating genetic gain using non-parametric procedures have not been developed.

Plot means from each geographic source were subjected to analysis of variance as follows:

Source	df	EMS
Rep	r-1	$\sigma^2_e + c\sigma^2_r$
Clones	c-1	$\sigma^2_e + r\sigma^2_c$
Rep x clones	(r-1)(c-1)	σ^2_e

Data were then subjected to analysis of variance over sources ignoring the restrictions of the triple lattice design as follows:

Source	df	EMS
Rep	r-1	$\sigma^2_{rs} + s\sigma^2_r$
Sources	s-1	$\sigma^2_{rs} + r\sigma^2_s$
Rep x sources	(r-1)(s-1)	σ^2_{rs}
Clones/source	(c-1)s	$\sigma^2_e + r\sigma^2_c$
Clones x rep/source	(c-1)(r-1)s	σ^2_e

Within source heritabilities were computed as:

$$h^2 = \frac{\sigma^2_c}{\sigma^2_c + \sigma^2_e}$$

The predicted genotypic gain (PGG) from selecting the best clones within each geographic source was computed as:

$$PGG_{c/s} = i \frac{\sigma^2_c}{\sqrt{\sigma^2_c + \frac{\sigma^2_e}{r}}}$$

where i is the standardized selection differential from Becker (1967).

Similarly, predicted genotypic gain from selecting the best sources was computed as:

$$PGG_s = i \frac{\sigma^2_s}{\sqrt{\sigma^2_s + \frac{\sigma^2_{rs}}{r}}}$$

and the predicted genotypic gain from selecting the best clones within the best sources is the sum of $PGG_{c/s}$ and PGG_s.

RESULTS

Leaf beetle damage was severe in the study area. The mean rating was 6.56 (on the 1 to 11 scale), indicating that an average of approximately one-half of the leaf area was destroyed. Source means varied from 4.98 to 7.58. Although Duncan's new multiple range test revealed few significant differences (0.05 level) among sources, northern sources appeared more tolerant than southern sources (Table 1). All but three of the 18 more northern sources had better than average tolerance. Many of the clones from the northern sources had ratings of 5 or less; many of the clones from the southern sources had ratings of 8 or greater.

A geographic pattern for tolerance to leaf beetle defoliation is not surprising, since this same group of clones displayed geographic trends for Septoria leaf spot resistance (Cooper and Filer 1976) and Melampsora rust resistance (Cooper and Filer 1977). The collective indication is that cottonwood along the lower Mississippi River is not a single freely intermating population in equilibrium. It may be that trees growing along the crumbling banks of the northern portions of the Mississippi River or some of its tributaries have floated down the river during spring floods and deposited seed of resistant genotypes, causing differences in tolerance observed among clones from the different geographic locations.

Heritability for tolerance to the leaf beetle was low (0.18) and standard errors were high, probably because of erratic distribution of insects in the 30-acre experimental area. Analysis of variance revealed significant differences among clones (.05 level of probability) in only 15 of 36 sources, and Friedman's Ranked Sign Test revealed significance in 13 of 36 sources.

This study will continue with a selection scheme designed to retain a moderate amount of genetic diversity. Choosing the best 9 of 36 sources should

result in a 3.5 percent genotypic gain in leaf beetle tolerance. Considering only the 9 best sources, selection of the 10 best clones per source should give 11.6 percent additional improvement. This group of 90 clones should have 15.1 percent more tolerance to the leaf beetle than the general population. More observations per clone would have permitted additional gain, but this would have been impractical with such a large number of clones.

Table 1.--Incidence of cottonwood leaf beetle damage to clones of different geographic origin.

Source	Latitude (N)	Mean score[1/]	Number of clones: Most resistant ≤4	Most resistant ≤5	Least resistant ≥8	Least resistant ≥9
1	34°57'	5.41 d-e	6	16	2	1
2	34°45'	6.68 a-e	0	4	5	1
3	34°43'	6.02 a-e	1	9	2	1
4	33°57'	6.04 a-e	1	8	2	1
5	33°57'	7.46 a-b	0	1	16	7
6	33°57'	7.29 a-c	1	4	17	3
7	33°46'	6.37 a-e	1	7	5	0
8	33°45'	6.19 a-e	3	11	5	1
9	33°35'	6.33 a-e	2	8	4	1
10	33°30'	6.17 a-e	1	8	3	1
11	33°06'	6.41 a-e	2	5	4	1
12	33°02'	4.98 e	12	21	1	1
13	32°52'	5.92 a-e	3	10	1	1
14	32°51'	6.03 a-e	1	7	4	0
15	32°45'	5.75 c-e	6	13	2	1
16	32°38'	6.49 a-e	0	5	6	1
17	32°16'	6.03 a-e	1	10	3	0
18	32°07'	6.29 a-e	5	9	6	3
19	32°05'	7.01 a-d	0	2	7	0
20	32°03'	6.65 a-e	0	4	6	3
21	31°59'	7.58 a	0	0	15	5
22	31°56'	7.22 a-c	0	1	11	1
23	31°52'	7.34 a-c	0	2	14	4
24	31°44'	6.55 a-e	1	5	5	1
25	31°40'	7.42 a-e	1	2	20	5
26	31°37'	6.86 a-d	1	3	5	1
27	31°31'	6.67 a-e	0	3	5	0
28	31°11'	7.21 a-c	0	2	13	2
29	31°10'	5.83 b-c	2	12	6	0
30	31°05'	6.40 a-e	1	8	5	2
31	31°01'	7.09 a-d	0	3	13	1
32	30°59'	6.99 a-d	0	4	10	5
33	30°37'	7.31 a-c	0	6	13	11
34	30°37'	6.56 a-e	0	4	5	1
35	30°36'	7.19 a-c	0	3	8	5
36	30°31'	6.57 a-e	0	2	4	2
Total			52	222	253	74
Mean		6.56				

[1/] Sources not sharing a common letter are different at the 5-percent level, Duncan's new multiple range test.

Since cottonwood can be easily asexually propagated, and leaf beetle damage can be scored during the first growing season, repeated cycles of testing are practical. The selected clones will be tested in 1977 and subsequent years to identify clones with consistently high tolerance to the cottonwood leaf beetle.

LITERATURE CITED

Arru, Giovanni M. 1975. Resistenza dei pioppi agli insetti secondo le osservazioni fatte in Italia negli anni 1930-72. Cellulosa e Carta 26: 45-49.

Becker, Walter A. 1967. Manual of procedures in quantitative genetics. 2nd ed. Pullman: Wash. State Univ. Press. 130 p.

Cooper, D. T. and Filer, T. H., Jr. 1976. Resistance to Septoria leaf spot in eastern cottonwood. Plant Dis. Rep. 60: 813-814.

Cooper, D. T. and Filer, T. H., Jr. 1977. Geographic variation in Melampsora rust resistance in eastern cottonwood in the Lower Mississippi Valley. 10th Cent. States For. Tree Improv. Conf. Proc. p. 146-151.

SESSION IV - POPULATION GENETICS

MODERATOR: JAMES BARKER

USE OF ISOENZYME TECHNIQUES IN FOREST GENETICS RESEARCH

M. Thompson Conkle and W. T. Adams[1/]

Abstract.--Genetic variation among loblolly pine (Pinus taeda L.) samples from a natural stand and among clones in seed orchards was analyzed using simply inherited isozyme markers. Alleles for eleven enzyme loci were found useful for genotyping trees in a natural stand in North Carolina. The pines were highly variable with as many as seven alleles per isozyme gene. On the average, close to 30 percent of the loci per tree were heterozygous. Similar levels of variability and heterozygosity were found among the clones in two seed orchards. Such variability makes it possible to uniquely identify most, if not all, of the clones in a seed orchard. Additional genes and other southern pine species are suited for similar analyses. A study with 15 different enzyme systems and samples from six species; loblolly, shortleaf, slash, pond, longleaf, and Virginia pines, suggested that as many as 26 different loci may be available for analysis and most loci appear to be similar for these species.

Additional keywords: Single genes, seed enzymes, conifers, seed orchards, Pinus taeda, P. echinata, P. elliottii, P. serotia, P. palustris, P. virginiana.

Ask a forest geneticist what could be accomplished if a variety of single gene markers were available and you are likely to hear research proposed that would keep armies of scientists busy for many years. The analysis of conifer enzymes yields data for numerous single gene markers.

Enzymes are primary gene products. Their molecular structure is determined by the DNA code of nuclear genes. Different DNA codes of a gene can alter the form of the specific enzyme produced. A technique called gel electrophoresis separates different forms of similar enzymes. The forms segregate and recombine as expected for Mendelian hypotheses.

The research results in this paper will be drawn from studies of southern pines with the principal emphasis on loblolly pine. In independent investigations, the authors analyzed seed from trees growing in a natural stand in the Schenck Forest, North Carolina State University, Raleigh, North Carolina (M. T. Conkle) and also from trees in two clonal-seed-orchards belonging to Champion International, Newberry, South Carolina (W. T. Adams). Our studies show that this technique can be extended to include more enzyme systems and other species of southern pines. The results will be presented after a short description of electrophoresis and seed sampling techniques.

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METHODS

Electrophoresis technique

Starch gel slabs (13% starch) are loaded with a row of paper wicks. Each wick carries a liquid sample from a seed. The samples contain a mixture of enzymes. Direct electric current is applied to gels, and enzymes with negative electric charges migrate toward the anode. Positively charged enzymes migrate toward the cathode. The rate of migration of any particular enzyme depends upon the size of the net electric charge on the molecule. The current is applied for a specific length of time or until marker dyes reach a specific distance. Thick gels are cut into several slices and each slice is stained for a different enzyme system. The bands that appear on the stained slices mark the relative migration distances of specific enzymes. In this manner, numerous seed samples can be analyzed for genes within several enzyme systems. For a more complete description of techniques, see Brewer (1970) and Conkle (1972).

Seed samples

Conifer seeds have excellent tissues for isoenzyme analyses. The female gametophyte, which is the nutrient tissue surrounding the embryo, is haploid (1N) and has the same genetic constitution as the female gamete, the egg. The analysis of several gametophytes from different seeds of a tree reveals the genotype of that tree. The enzyme bands for a particular allele consistently migrate to the same location on gels. A tree that is homozygous for a gene will have bands from gametophytes in only one location on the gel. A tree heterozygous for alleles of a gene will have bands in two locations. Heterozygotes are tested to determine whether the two different forms segregate with a 1:1 ratio.

The genotype of the embryo (2N) also can be determined for several enzymes. Knowing the genotype of an embryo and its adjacent gametophyte, the pollen contribution to the embryo can be inferred. Thus, the parent tree genotype, and the genotypes of individual eggs and pollens can be determined by the analysis of seed from a single tree.

Dry seed-samples can be used, but pine seeds that are just beginning to germinate give darker staining bands (Conkle, 1971) and are easier to prepare. The data presented in the remainder of this paper are based on the analysis of six gametophytes for the determination of each tree's genotype. Where several species were compared, 24 seeds from general seed collections for each species were analyzed.

RESULTS

Isozymes from loblolly pines in a natural stand

The locations and ages of the 146 cone bearing trees sampled were recorded and open-pollinated cones of each tree were collected. The seeds of each tree were analyzed for seven different enzymes systems (ACPH, ADH,

BANA, EST, GOT, LAP, PGM; see [2/] for the full names of enzymes, these abbreviations will be used throughout the paper). One gene with two or more alleles was identified in the ACPH, ADH, BANA, and PGM enzyme systems. Two genes were active in LAP and EST systems (EST-1, EST-2, LAP-1, and LAP-2). Three genes were scored in the GOT system (GOT-1, GOT-2, and GOT-3). In all, each tree was analyzed for genotypes at 11 different loci.

All 11 loci segregated for two or more alleles and the segregation of alleles from the gametophytes of heterozygous trees did not differ significantly from the expected 1:1 ratio. Nine of the 11 loci had 3 or more alleles within the stand (table 1). The gene with the greatest number of alleles was ACPH with 7 distinctly different allelic forms.

The genotypes of all 146 trees were used to estimate the allele frequencies for the stand (table 1). Each tree has two alleles per gene and the frequency estimates are based on a total of 292 observations.

Table 1.--Allele frequencies for eleven genes from trees of a natural stand of loblolly pine near Raleigh, North Carolina

Gene	Allele number 1	2	3	4	5	6	7
	Frequency						
ACPH	.82	.05	.05	.03	.03	.01	.01
ADH	.99	.01					
BANA	.92	.04	.04				
EST-1	.40	.31	.11	.08	.07	.03	
EST-2	.64	.34	.01	.01			
GOT-1	.77	.20	.03				
GOT-2	.51	.44	.05				
GOT-3	.79	.21					
LAP-1	.87	.05	.04	.04			
LAP-2	.53	.46	.01	a/			
PGM	.95	.03	.02				

a/ Rare allele found in only one tree.

The trees of the stand are genetically highly variable. Some genes have one allele in high frequency. For example, ACPH, ADH, BANA, LAP-1, and PGM have one allele that occurs greater than 80 percent of the time.

2/ ACPH = acid phosphatase, ADH = alcohol dehydrogenase, BANA = endopeptidase, CAT = catalase, EST = esterase, GDH = glutamate dehydrogenase, GOT = glutamate oxaloacetate transaminase, G-6-PDH = glucose-6-phosphate dehydrogenase, IDH = isocitrate dehydrogenase, LAP = leucine amino peptidase, MDH = malate dehydrogenase, PER = peroxidase, 6-PGDH = 6-phosphogluconate dehydrogenase, PGI = phosphoglucoisomerase, PGM = phosphoglucomutase, TO = tetrazolium oxidase (indophenol oxidase).

Other genes, such as EST-1, EST-2, GOT-1, GOT-2, GOT-3, and LAP-2, have two or more alleles with intermediate frequencies. All the genes, except GOT-3, have rare alleles that occur at frequencies of 5 percent or less.

This natural stand was chosen because two age classes could be identified. A group of 48 older trees are the suspected parents of 98 younger trees that sample the regeneration of an old field. The gene frequencies for the two age classes and the geographic distribution of genes over the stand are the subjects of future analyses. Preliminary observations are that the allele frequencies are approximately equal in the older and younger trees, and that genotypes are randomly distributed within the stand.

The isozyme data was used to estimate the degree of heterozygosity present in the stand. The number of heterozygous loci per tree was counted. On the average, trees were heterozygous for 3.5 of the 11 genes; --heterozygosity is estimated to be 32 percent. A large number of trees were heterozygous at 2 to 5 loci. Only one tree was homozygous for all 11 loci. On the other end of the scale, one tree was heterozygous for 7 loci and another was heterozygous for 8. These estimates are comparable to those reported for other pine species (Feret 1974, and Rudin et al. 1974).

The uniqueness of each tree's genotype is related to the number of alleles per locus and their frequency in the population. Each gene with multiple alleles has $n(n+1)/2$ possible genotypic classes, where n = the number of alleles. The ACPH gene with 7 different alleles could produce 28 different classes of diploid genotypes. If this line of reasoning is extended to include all 11 loci, there could be 10 genotypic classes for LAP-1, 10 classes for LAP-2, and so on. The total number of different diploid genotypes that are possible for these genes, is the product of the number of genotypic classes for each locus ($28 \times 10 \times 10 \times \ldots$). For these 11 genes 6,858,432,000 different genotypes are possible. If the proportions found for the most frequent genotypic class of each locus are multiplied together, the most frequently duplicated 11-locus genotype would be expected to be found in only 3 out of 1,000 individuals. Various factors such as the breeding system, family relationships, linkage, selection, and genetic drift can alter this expectation, but the point is that each tree is virtually a unique genotype.

Genetic identity of clones in loblolly pine seed orchards

The large amount of genetic variability found in the natural stand leads to the expectation that many of the twenty to thirty clones of a seed orchard should be uniquely identifiable on the basis of their isozyme genotypes. The analysis of seed from trees in two orchards shows that most clones have unique genotypes (table 2).

The uniqueness of clone genotypes in orchards (table 2) is based on the analysis of 10 loci from six enzyme systems (GDH, GOT, LAP, 6-PGDH, PGI, and PGM). Twenty six of the 27 clones in the HIGH SPECIFIC GRAVITY orchard have unique 10-locus genotypes. A similar result was found for the clones in the LOW SPECIFIC GRAVITY orchard (22 of 23 clones have unique genotypes). When the genotypes of clones from both orchards are considered together, 47 of the total of 50 clones are unique. Thus, not only are most clones within an

orchard unique; --virtually all clones in the two orchards have unique genotypes. Only one genotype is common to a clone in each orchard.

Table 2.--Average heterozygosity and genotypic uniqueness of clones in two loblolly pine seed orchards

Seed orchard	Number of clones	Average heterozygosity per clone (Percent)	Number of unique genotypes
HIGH SPECIFIC GRAVITY CLONES	27	27	26
LOW SPECIFIC GRAVITY CLONES	23	29	22

The high degree of variability among clones in these loblolly orchards agrees with data supplied by Ms. Serena Hunter, North Carolina State University, Raleigh (personal communication, May, 1977). She finds all 27 clones in a Weyerhaeuser Company orchard, Washington, North Carolina, to be uniquely identifiable using twelve genes from six enzyme systems (ACPH, EST, GOT, LAP, MDH, and 6-PGDH).

The estimates of average heterozygosity per clone for the two orchards (table 2) are close to the 32 percent value found for the native stand and Ms. Hunter's estimate of 32 percent for clones in the North Carolina seed orchard. Although these individual estimates are based on different loblolly pine samples and different enzyme loci, they cluster around a common value of 30 percent.

Isozymes from six southern pine species

Loblolly, shortleaf, slash, pond, longleaf, and Virginia pines were analyzed using the same techniques but including more enzyme systems. The goal of this study was to identify more enzyme systems and extend the analysis to other southern pines. Our interest centers upon estimating the number of potentially useful loci.

Each of the species was represented by a small number of seeds (24 seeds per species). The seeds were samples from large open-pollinated collections. The female gametophyte and the embryo of each seed were analyzed separately. In all, each tissue was examined for isozymes in 15 different enzyme systems (ACPH, ADH, CAT, EST, GDH, GOT, G-6-PDH, IDH, LAP, MDH, PER, 6-PGDH, PGI, PGM, and TO).

All 15 enzyme systems resolve some bands for gametophytes and embryos of the six southern pine species that were included. The first noteworthy observation is that there is a striking similarity between the patterns of enzyme bands for all species within each enzyme system. The number of bands, their migration distances, their characteristic sizes and stain intensities all indicate that similar if not identical genes are acting within the six

species. Similar findings were reported for a comparison of pitch and loblolly pines (Adams and Coutinho, 1977). It seems safe to predict that differences between these species will be a function of the allele frequencies that are found for a set of genes common to all southern pines.

The enzyme bands of the various species are so similar that inferences from genetic studies of loblolly pine might apply to the other species. First, using data from gametophytes only, at least one locus may be available from the ACPH, CAT, GDH, G-6-PDH, and PGM enzyme systems. Two loci may be available from each of the following systems; ADH, EST, LAP, 6-PGDH, PGI, and TO. It may be possible to obtain information on as many as three loci from the GOT, MDH, and PER enzyme systems. Thus, current techniques for isozyme analysis of southern pines may be capable of providing genotypic information for as many as 26 loci. This is more than twice the number of loci that were used for the current analyses of trees in the natural stand and clones in seed orchards.

In general, the enzyme bands from embryos of all six species were not as well resolved as were the corresponding bands for gametophytes. Despite this fact, embryos yielded some bands in most enzyme systems for all species. However, PER enzymes from embryos, were poorly resolved. Embryos stained for ADH were best resolved for loblolly and longleaf pine samples. The IDH bands from longleaf and slash pine embryos were resolved, other species stained poorly. With these exceptions, it appears likely that embryo genotypes will be available for loci in several enzyme systems. Many of the bands in embryos appear to be expressing genes that are common to gametophytes.

DISCUSSION

Much work in the area of population genetics of forest trees has been based upon the statistical partitioning of the total variation in phenotypic traits into causes that are related to genetics and causes that are related to test environments. In the majority of such studies, the observed response cannot be attributed to the action of a specific gene and the response is often related to the specific test conditions. In contrast, isozyme techniques identify alleles for specific loci and the identification of the tree genotype does not depend upon the test environment.

Trees may have as many as 100,000 different kinds of protein and the effect of a single enzyme variant is likely to be unmeasurable. But enzymes can serve to estimate the genetic variability within and between groups of trees.

A concern that forest researchers have voiced is whether sufficient genetic variability is present to characterize individual forest trees, forest tree populations, and species. Another concern has been whether the number of enzyme loci analyzed is sufficiently large to be considered an adequate sample. Finally, forest researchers are asking whether the enzyme loci that can be analyzed are independent samples of a tree's genes.

Loblolly pines have large amounts of genetic variability judging from these enzyme studies. On the average, we find approximately 3.75 alleles per locus for the genes reported in this paper. Loblolly pines are heterozygous

at nearly 30 percent of the loci that were sampled. As a consequence, an enormous number of different tree genotypes is possible and each tree is virtually a unique genotype. This genotypic uniqueness was shown useful for identifying individual clones within seed orchards. In the future, the technique may be used to determine how variability is distributed among samples from natural populations of southern pines and it may prove useful for seed source identification. The technique may also be used to compare trees in tree improvement programs and to follow the genetic variability of these selected populations through future cycles of selection.

The current study also attempted to expand the number of enzyme systems that are appropriate for the analysis of southern pines. There are preliminary indications that at least 15 enzyme systems are capable of resolving bands and each enzyme system may resolve between one and three loci. It appears that current technology could provide analyses for about 26 loci. Since this aspect of the work has not been thoroughly researched, we conclude that this is a minimum estimate and future analyses should increase the number of usable loci.

The technique is appropriate for many southern pines, namely; loblolly, shortleaf, slash, pond, longleaf, and Virginia pines. Seeds from these various species resolved enzyme bands with similar characteristics. The loci of the various species appear to be comparable. Future studies of speciation, and introgression will depend upon estimating the allele frequencies for genes common to the different species.

Though no linkage studies for southern pines were reported in this paper, it should be pointed out that they are planned. The haploid gametophyte of conifer seeds may promote conifers as the best possible materials for such studies. The test of independent assortment of alleles for various loci can be done using the female gametophytes of a tree heterozygous for several loci. Since pollen genotypes can be determined, linkage studies are also possible using data from male gametes but selfed-families or full-sib families are required.

Other studies are possible using the information from alleles in pollen. Investigations of outcrossing and selfing (i.e.; Muller, 1976), studies of hybridization (i.e.; Adams and Coutinho, 1977, and Tobolski and Conkle, 1977) and studies of pollen dispersion, migration, and effectiveness are a few examples. Recall, also, that numerous loci in the loblolly pine natural stand had alleles occurring with low frequencies. These rare alleles may prove very useful as markers in pollen studies.

The research and thoughts concerning future research presented in this paper are but a sampling of the potential this technique holds for investigating genetic variability in forest trees.

LITERATURE CITED

Adams, W. T., and S. Coutinho. 1977. Isoenzyme genetic markers useful for studies of the Pinus rigida X P. taeda hybrid. Northeast For. Tree Improv. Cong. Proc. 24:1-13.

Brewer, G. J. 1970. An introduction to isozyme techniques. Academic Press, Inc. New York.

Conkle, M. T. 1971. Isozyme specificity during germination and early growth of knobcone pine. Forest Sci. 17(4):494-498.

_____. 1972. Analyzing genetic diversity in conifers... isozyme resolution by starch gel electrophoresis. USDA For. Serv. Res. Note PSW-264, p. 1-5.

Feret, P. P. 1974. Genetic differences among three small stands of Pinus pungens. Theor. and Appl. Genet. 44:173-177.

Muller, G. 1976. A simple method of estimating rates of self-fertilization by analyzing isozymes in tree seeds. Silvae Genet. 25(1):15-17.

Rudin, D., G. Eriksson, I. Ekberg, and M. Rasmuson. 1974. Studies of allele frequencies and inbreeding in Scots pine populations by the aid of the isozyme technique. Silvae Genet. 23(1-3):10-13.

Tobolski, J. J., and M. T. Conkle. 1977. Enzyme identification of Austrian X Japanese red pine hybrids in seed from mixed pollen controlled crosses. 10th Central States State For. Tree Improv. Conf. Proc., Sept. 22-23, 1976, Purdue Univ., West Lafayette, Indiana.

USE OF MONOTERPENE COMPOSITION IN FOREST GENETICS RESEARCH WITH SLASH PINE

A. E. Squillace[1/]

Abstract.--The concentrations of 4 of the 5 major monoterpenes in cortical oleoresin of slash pine are controlled by single genes, with high being dominant or partially dominant over low amounts. Environmental effects are small. Large differences occur between trees, and examples of 15 of the 16 possible phenotypes have been found. Distinctive patterns of geographic variation occur for each of the 4 monoterpenes shown to be simply inherited, with clinal trends being a dominant feature over much of the species range. Such detailed knowledge of variation and inheritance permits use of monoterpenes as gene markers for studying genetic problems. Uses include identifying relatives and seed origin and determining the degree of selfing and of wild pollen contamination in seed orchards.

Additional keywords: Pinus elliottii Engelm., essential oils, turpentine.

Interest in monoterpene composition at our laboratory originated from our research to develop strains of slash pine (Pinus elliottii Engelm.) that would yield large amounts of oleoresin for gum naval stores. The monoterpenes occurring in this species vary greatly in value, and the original objective of this monoterpene research, beginning in 1961, was to increase yield of the most valuable component, β-pinene, (Squillace and Fisher 1966). This work was fruitful, but we also soon learned that monoterpene composition could help solve other tree breeding problems. Hence, monoterpene composition became a major part of our research program. In this paper I briefly summarize findings on the variation and inheritance of monoterpene composition in slash pine and give some examples of how we use this information in our genetics research.

INDIVIDUAL TREE VARIATION AND INHERITANCE

The oleoresin of slash pine consists of about 20 percent monoterpenes, the remainder being mainly resin acids. The monoterpene fraction of oleoresin from xylem tissue consists mostly of α-pinene, β-pinene, and β-phellandrene. Frequently camphene, myrcene, α-phellandrene, and limonene occur as minor consitiuents, while traces of Δ-3-carene, and γ-terpinene occur occasionally. In oleoresin from cortical tissue of branch tips, the same constituents occur but the amounts of myrcene and/or limonene can be very high in some trees.

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The oleoresin of stem xylem is of greatest commercial importance, but the oleoresin from cortical tissue has proved to be the most useful for genetics studies. The composition of monoterpenes in stem xylem oleoresin often varies with height in the tree and depends partly upon distance from the live crown (Roberts 1970 and Franklin 1976). This effect tends to complicate sampling procedures for stem xylem oleoresin. The oleoresin in cortical tissue, on the other hand, is relatively constant within the crown. This desirable feature, plus the fact that cortical oleoresin has 5 major constituents compared to 3 in xylem oleoresin, has lead us to favor cortical oleoresin for genetics studies. Monoterpene composition in oleoresin of needles tends to be similar to that of branch cortical tissue. However, we usually use the latter, because of its relative ease of collection--most trees readily exude a droplet of oleoresin sufficient for analysis when branch tips are excised. Occasionally it is necessary to concentrate samples (Goodwin 1977).

The relative amounts of most of the monoterpenes are usually either high or low. That is, frequency distributions for oleoresin from a large number of trees are usually bimodal (figures 1A and B). One complication is that the location of modes can be affected by the presence or absence of other major constituents. For example, the mode for high β-pinene is lower in trees containing high limonene (fig. 1A) than in trees containing low limonene (fig. 1B). After studying over 2000 trees, we developed the classification scheme given in table 1 for 4 of the major constituents. Since clear evidence of bimodality for α-pinene is lacking, this constituent is excluded from table 1.

Figure 1.--Frequency distributions for β-pinene. (From Gansel and Squillace 1976).

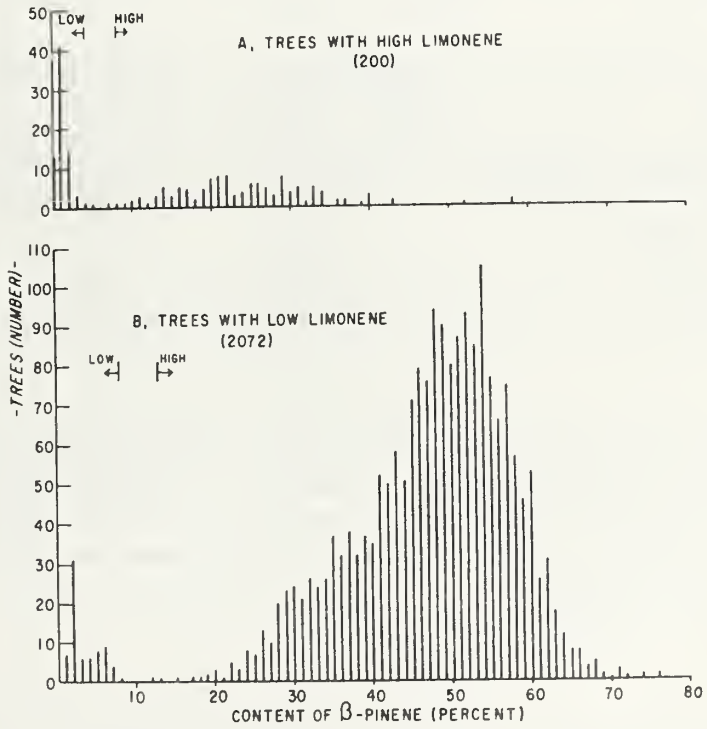


Table 1.--Criteria used for classifying trees as having high or low amounts of each monoterpene. (From Gansel and Squillace, 1976)

Monoterpene	Low	High	Constraint condition
	--percent--		
β-pinene	0-4	8+	High limonene
	0-8	12+	Low limonene
Myrcene	0-6	9+	High limonene
	0-4	7+	Low limonene
Limonene	0-8	16+	None
β-phellandrene	0-2	4+	None

Bimodality in the frequency distribution for a monoterpene suggests that the relative amount of it is controlled by a single gene with dominant gene action. Studies of parents and their self- and cross-pollinated offspring show that this is indeed the case for all constituents showing clear bimodality (table 2) (Squillace 1971, 1976a). Curiously, high was found to be dominant over low in all cases.

As the work progressed we were able to identify enough genotypes to study the degree of dominance expressed by monoterpenes. Preliminary indications are that dominance is partial rather than complete in most cases (table 3). In cases of incomplete dominance we may eventually be able to distinguish heterozygotes from homozygotes, enhancing the utility of monoterpenes as gene markers.

Environmental effects on monoterpene composition of cortical oleoresin of slash pine are small (Squillace and Fisher (1966) and Gansel and Squillace (1976)).

Since 4 of the major monoterpenes show bimodality, we can classify trees into 16 phenotypes as indicated in table 4. Note that many of the trees fall into a few of the phenotypic classes, but appreciable numbers occur in other classes. Only one class lacks representatives. Note especially that two trees occur which, lacking genes for high amounts of the monoterpenes known to be simply inherited, are almost entirely α-pinene. At the other extreme, some trees contain the high allele for all 4 monoterpenes.

GEOGRAPHIC VARIATION

In one of our studies, we sampled trees originating from all portions of the species range. Trees were classified as having either high or low amounts of each of the 4 monoterpenes shown to be simply inherited. Distinctive patterns occurred. For example no trees having high β-pinene were

Table 2.--Segregation data for inheritance of four monoterpenes in branch cortical oleoresin of slash pine.

Type of mating	Families	Individuals			
		Observed		Expected	
		High	Low	High	Low
		Number			
		β-pinene			
BB x --	78	980	0	980.0	0.0
Bb x Bb	8	86	25	83.2	27.8
Bb x bb	2	9	10	9.5	9.5
bb x bb	0	---	--	-----	----
		Myrcene			
MM x --	2	32	0	32.0	0.0
Mm x Mm	17	151	44	146.2	48.8
Mm x mm	45	278	330	304.0	304.0
mm x mm	26	1	278	.0	279.0
		Limonene			
LL x ll	1	31	0	31.0	0.0
Ll x Ll	0	--	--	----	---
Ll x ll	2	14	19	16.5	16.5
L- x ll	1	2	0	$\geq$ 1.0	$\leq$ 1.0
Ll x wind	1	7	4	$\geq$ 5.5	$\leq$ 5.5
ll x ll	90	0	1052	.0	1052.0
		β-phellandrene			
PP x --	63	769	14	783.0	0.0
Pp x Pp	8	138	48	139.5	46.5
Pp x pp	17	43	49	46.0	46.0
pp x pp	2	1	50	.0	51.0

found in extreme south Florida (fig. 2). From this point the percentage of such trees increased rapidly to the north. A plateau was reached where all trees had high β-pinene. Clinal patterns, with plateaus in some cases were also found for other monoterpenes (figs. 3 to 5).

UTILITY OF MONOTERPENE COMPOSITION

Identification of Seed Origin

The geographic patterns of variation in monoterpene composition offer

Table 3.--Degree of dominance for simply-inherited monoterpenes in slash pine.

Phenotypic group[1]	Homozygous dominants		Heterozygotes		Homozygous recessives		Degree of dominance[2]
	Basis, trees	Average content	Basis, trees	Average content	Basis, trees	Average content	
	No.	%	No.	%	No.	%	
β-pinene							
BM&P	156	47.0	12	44.3	60	3.1	0.88
BmLP	1	29.0	25	22.8	28	1.1	.56
Bm&P	257	51.0	8	39.2	9	5.7	.48
Wtd. Ave.[3]							.63
Myrcene							
BM&P	2	34.5	228	21.7	1076	1.0	.24
Limonene							
BMLP	1	37.0	11	38.4	548	.5	1.08
bmLP	1	75.0	5	70.0	9	.3	.87
bmLp	1	89.0	1	89.0	2	.0	1.00
Wtd. Ave.[3]							1.01
β-phellandrene							
BM&P	168	18.6	61	11.5	83	.4	.22
Bm&P	183	16.4	24	11.3	269	.5	.36
bmLP	1	33.0	1	14.0	3	.0	-.15
Wtd. Ave.[3]							.28

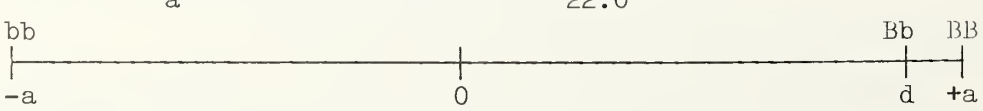
1/B, M, L, and P represent high amounts of β-pinene, myrcene, limonene, and β-phellandrene, respectively, while lower case letters represent low amounts.

2/Computed by methods outlined in Kempthorne (1957, p. 373). For example, the degree of dominance for β-pinene group BM&P was computed as follows:

$$a = \frac{47.0 - 3.1}{2} = 22.0$$

$$d = 22.0 - (47.0 - 44.3) = 19.3$$

$$\frac{d}{a} = \text{degree of dominance} = \frac{19.3}{22.0} = .88$$



3/Weighted by number of homozygous dominants and heterozygotes involved.

Figure 2.--Percent of trees having high β-pinene (From Gansel and Squillace, 1976).

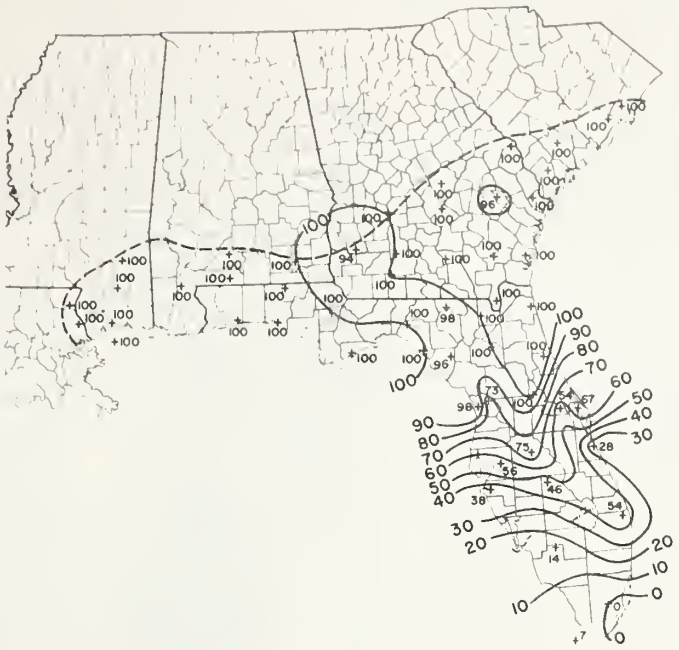


Figure 3.--Percent of trees having high myrcene (From Gansel and Squillace, 1976).

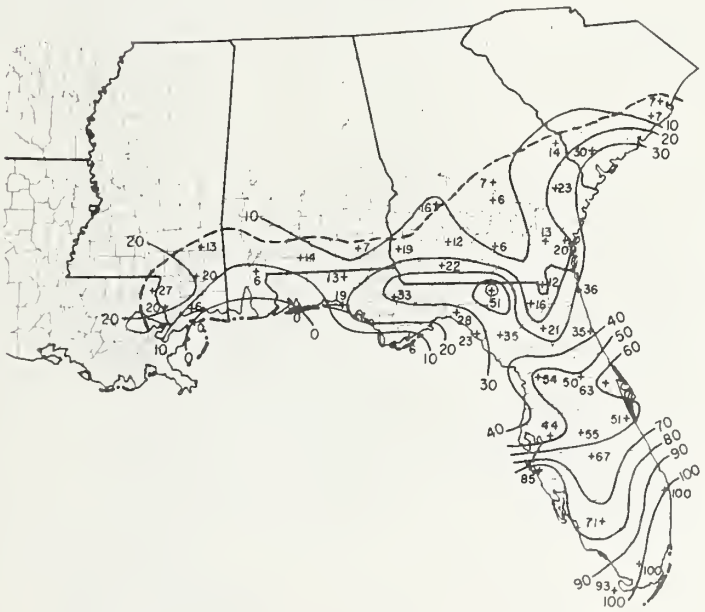


Figure 4.--Percent of trees having high limonene (From Gansel and Squillace, 1976).

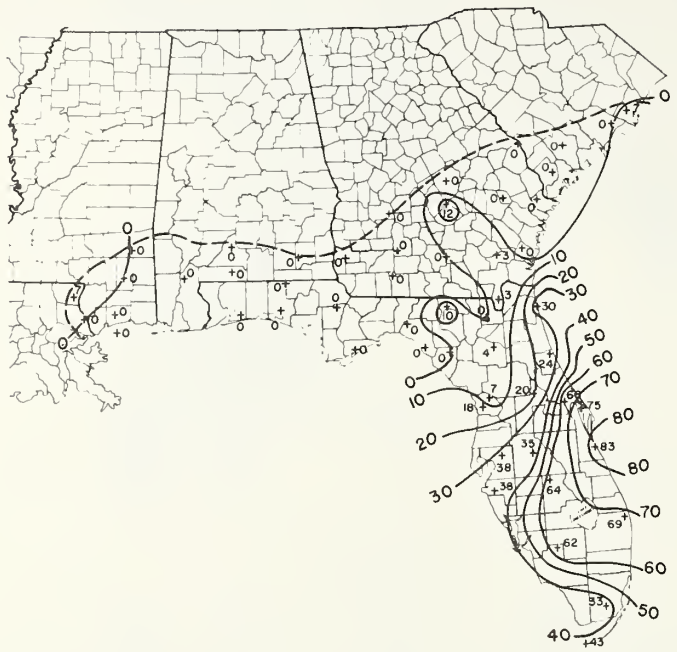


Figure 5.--Percent of trees having high β-phellandrene (From Gansel and Squillace, 1976).

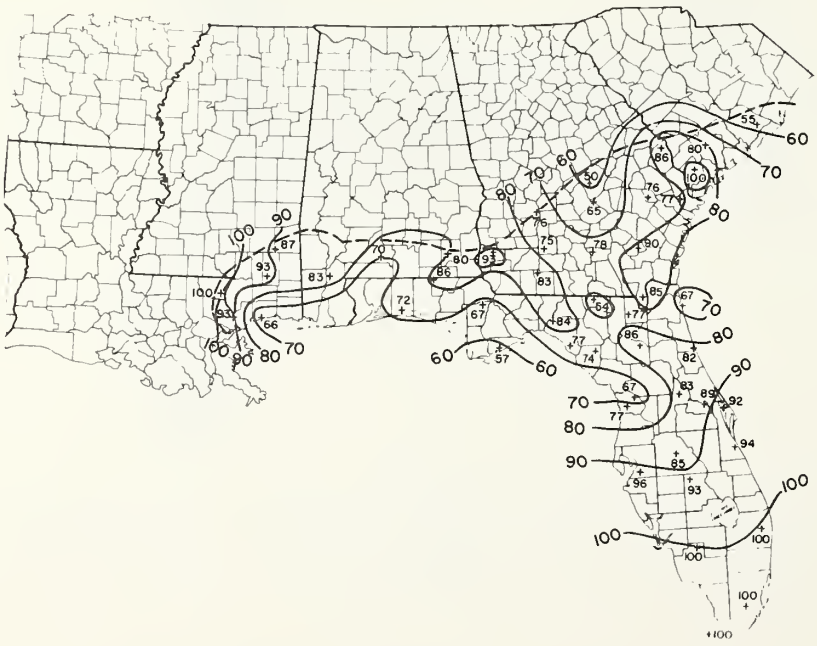


Table 4.--Average monoterpene composition in branch cortical oleoresin of 15 slash pine phenotypes. 1/

Phenotype 2/	Basis, trees	Composition 3/				
		α-pinene	β-pinene	Myrcene	Limonene	β-phellandrene
	Number	Percent				
BMLP	32	8.4	18.9	14.9	44.3	13.4
BMLp	6	11.8	21.2	15.8	49.8	1.3
BMℓP	548	17.2	42.5	23.7	.5	15.8
BMℓp	83	29.3	44.3	25.5	.1	.4
BmLP	66	10.3	25.3	2.5	52.0	9.8
BmLp	10	19.1	27.0	2.4	50.5	.9
BmℓP	1076	34.5	49.2	1.0	.6	14.2
Bmℓp	269	46.1	52.2	.6	.3	.5
bMLP	40	6.2	1.2	18.6	53.0	20.8
bMLp	0	-	-	-	-	-
bMℓP	60	16.8	3.1	38.9	.9	39.6
bMℓp	1	44.0	1.0	55.0	.0	.0
bmLP	28	8.5	1.1	4.0	71.6	14.7
bmLp	3	8.7	1.0	4.0	86.3	.0
bmℓP	9	60.0	5.7	.9	.3	31.9
bmℓp	2	96.0	3.5	.0	.0	.0

1/ Sixteen phenotypes are theoretically possible, but no trees of the type bMLp occurred in the sample of 2233 trees.

2/ B, M, L and P represent high amounts of β-pinene, myrcene, limonene, and β-phellandrene, respectively, while lower case letters represent low amounts.

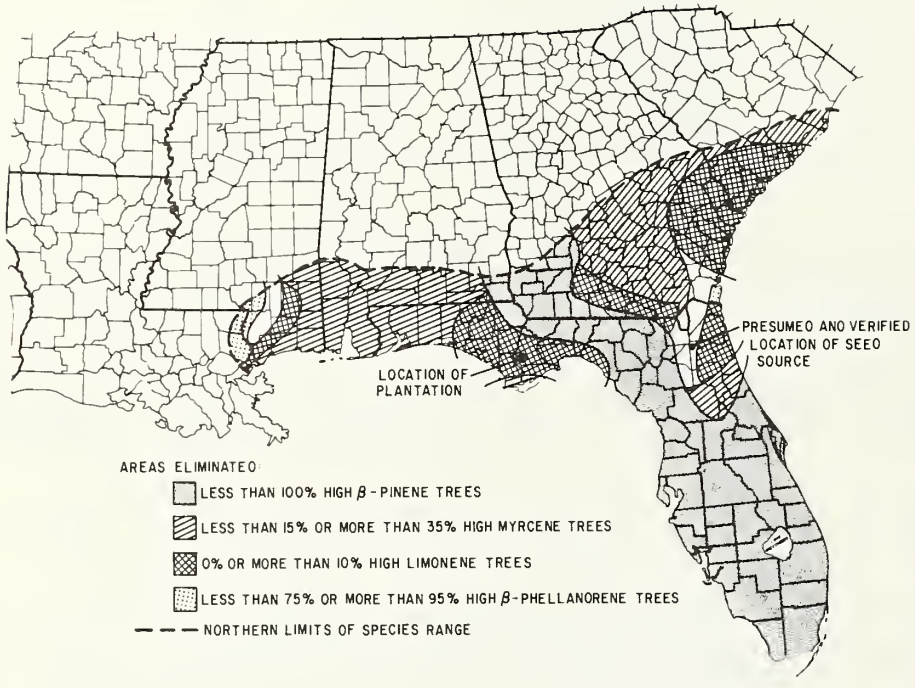
3/ Small amounts of camphene and/or α-phellandrene frequently occur.

possibilities for identifying the approximate geographic origin of seed used in plantations of unknown origin. We recently had an opportunity to test the procedure on 3 plantations that were planted in west Florida in about 1936. On the basis of uncertain records, the seed were believed to have come from several counties in northeast Florida. We sampled 30 trees in each plantation and concluded that the seed used for each were of roughly the same geographic origin (table 5). Hence, we used the averages to estimate their geographic origin. Taking each of the 4 chemicals successively, we eliminated areas where the seed could not likely have originated (fig. 6). All but two areas were eliminated, a small one in southeast Mississippi and a larger one in northeast Florida and southeast Georgia. Hence, the analysis suggested that the purported origin given in the plantation records, northeast Florida, was correct. Possibilities also exist for identifying seed orchard seed.

Table 5.--Percentages of trees having high amounts of 4 monoterpenes in 3 adjacent slash pine plantations.

Plantation	β-pinene	Myrcene	Limonene	β-phellandrene
A	100	23	3	87
B	100	13	3	87
C	100	37	7	80
Average	100	24	4	85

Figure 6.--Determination of probable origin of seed in a 40 year old slash pine plantation. (See table 3)



Identification of Relatives

Knowledge of the mode of inheritance of 4 major monoterpenes permits us to identify relatives in trees to about the same extent that relatives can be identified in human beings using blood types. Identification of ramets within clones is, of course, done rather easily. In several instances we have suspected that certain ramets had been mislabelled, on the basis of cone and seed characteristics, and then verified such suspicions by examining monoterpene composition. In some instances we were able to determine the proper clone. Identification of parents and progenies is, of course, less certain although in one instance we detected and corrected a serious labelling error in a progeny test.

Selfing and Wild Pollen Contamination in Orchards

An unusual situation in one of our demonstration seed orchards permitted us to make rough estimates of both the extent of selfing and the degree of wild pollen contamination. The orchard covered 5 acres (2 hectares) and all slash pine trees within 400 feet (122 meters) had been removed. It contained 9 clones, which were all related as either half sibs or full sibs. We knew monoterpene genotypes of all the clones (table 6). One of the clones, No. 5, was thought to be suitable for estimating the degree of selfing because 1/16 of its selfed progeny would be of the type bmℓp, which could not be produced from matings among clones in the orchard. Hence, wind-pollinated seeds were collected from it and monoterpene composition was determined on seedlings grown from them.

Table 6.--The genotypes of nine clones in an experimental slash pine orchard being used to estimate selfing and wild pollen contamination.

Clone number	Genotype
1	BB Mm ℓℓ Pp
2	BB Mm ℓℓ Pp
3	BB mm ℓℓ Pp
4	BB Mm ℓℓ Pp
5	Bb mm ℓℓ Pp
6	BB Mm ℓℓ Pp
7	BB mm ℓℓ PP
8	Bb mm ℓℓ PP
9	BB Mm ℓℓ PP

Frequencies of the various phenotypes showed that 1.3 percent were of the type bmℓp (table 7). Hence, we could guess that approximately 16 x 1.3 = 21 percent of the progeny of clone 5 were selfs. However, we also computed expected frequencies, first assuming that all matings were crosses among trees in the orchard, and then also by assuming that all progeny were sired by contaminate pollen. The latter were made on the basis of known gene frequencies determined for the region in which the orchard was located.

Comparison of observed and expected frequencies strongly suggested that considerable wild pollen contamination is occurring in this orchard. All seedlings containing high limonene plus those of the type bMℓP and bMℓp are necessarily contaminants because they cannot be formed by any mating among orchard clones. Also, the correlation of observed frequencies with frequencies expected from orchard out-crosses is smaller than with frequencies expected from orchard contamination. Note also that bmℓp trees can be produced by contamination as well as by selfing. Hence, a better estimate of the

Table 7.--Observed and expected phenotypic frequencies in 546 wind-pollinated progeny [1/] of clone 5 in an experimental slash pine orchard average.

Phenotype	Observed frequencies [1/]	Expected frequencies if pollen is entirely: Self	Orchard outcross	Wild (contamination)
BMLP	0.000	0.0	0.0	0.002
BMLp	.000	.0	.0	.001
BMℓP	.106	.0	.253	.095
BMℓp	.017	.0	.065	.031
BmLP	.011	.0	.0	.013
BmLp	.002	.0	.0	.004
BmℓP	.578	.562	.569	.615
Bmℓp	.220	.188	.077	.203
bMLP	.000	.0	.0	.000
bMLp	.000	.0	.0	.000
bMℓP	.009	.0	.0	.004
bMℓp	.004	.0	.0	.001
bmLP	.000	.0	.0	.000
bmLp	.000	.0	.0	.000
bmℓP	.040	.188	.036	.023
bmℓp	.013	.062	.0	.008
Sums	1.000	1.000	1.000	1.000

[1/] Averages of data from seed collections made in 1973 and 1974.

degree of selfing might be 16(1.3 - .8) = 8.4 percent and this agrees more closely with estimates of selfing that we have obtained in other orchards through use of chlorophyll-deficient seedlings as gene markers.

Thus, we believe that production of selfed seedlings in this orchard is low, but that contamination is high. Of course, these results apply only to one orchard, but they demonstrate the possible utility of monoterpene composition.

DISCUSSION

Several other uses of monoterpene composition in solving forestry problems have been suggested, as summarized by Squillace (1976b). For example, relationships have been shown between monoterpene composition and insect and disease resistance. Such correlations permit indirect genetic selection for these traits. Monoterpene composition is well suited for studies of relationships between species and for identification of hybrids. A recent paper (Squillace, et al. 1977) suggests gene flow from Caribbean pine (P. caribaea Mor.) into slash pine based on population analyses of monoterpene composition.

Utility of monoterpene composition will likely be enhanced by more modern gas chromatographs. A recent model, for example, provides for automatic injection of 35 samples, permitting around-the-clock analyses with a minimum of attendance. Thus, more intensive and extensive sampling will be feasible.

In short, monoterpene composition has proved to be very useful in many forest genetics studies and its use is likely to increase.

LITERATURE CITED

Franklin, E. C. 1976. Within-tree variation of monoterpene composition and yield in slash pine clones and families. For. Sci. 22:185-191.

Gansel, Charles R. and A. E. Squillace. 1976. Geographic variation of monoterpenes in cortical oleoresin of slash pine. Silvae Genet. 25(5/6).

Goodwin, Clifford L. 1977. Concentrating dilute oleoresin samples of monoterpenes by gas chromatography. USDA For. Serv. Res. Note SE-243, 2p.

Kempthorne, O. 1957. An introduction to genetic statistics. John Wiley and Sons, Inc. N. Y. 545 pp.

Roberts, Donald R. 1970. Within-tree variation of monoterpene hydrocarbon composition of slash pine oleoresin. Phytochemistry 9:809-815.

Squillace, A. E. 1971. Inheritance of monoterpene composition in cortical oleoresin of slash pine. For. Sci. 17:381-387.

Squillace, A. E. 1976a. Biochemical genetics and selection--composition of volatile terpenes. Presented at Meeting of IUFRO Working Parties on Forest Genetics, June 1976. Bordeaux, France.

Squillace, A. E. 1976b. Analyses of monoterpenes of conifers by gas-liquid chromatography. In: Modern Methods in Forest Genetics. Ed. J. P. Miksche. Springer Verlag, New York, Chapt. 6. (pp 120-157) 288 p.

Squillace, A. E. and Gordon S. Fisher. 1966. Evidences of the inheritance of turpentine composition in slash pine. USDA For. Serv. Res. Paper NC-6-53-60.

Squillace, A. E., D. G. Nikles and L. C. Saylor. (1977) Monoterpene composition in cortical oleoresin of *Pinus caribaea* and relation to *P. elliottii* of Florida. Preprint of paper for Third World Consultation on Forest Tree Breeding, Canberra, Australia, 1977.

USE OF MUTANTS IN FOREST GENETICS RESEARCH

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Abstract.-- Numerous genetic abnormalities (mutants) affecting pigmentation, development, and form have been described for the southern pines. Many of these traits behave as simply inherited Mendelian traits when produced by selfing and crossing. Such traits can often be used as genetic markers to trace pollen distribution, to test relative effectiveness of various pollens, or to make inferences about the genetic structure of stands or other populations such as seed orchards.

Additional keywords: Mendelian traits, particulate inheritance, pollen distribution, genetic structure of populations, seed orchards.

POPULATION GENETICS

Study of population genetics is directed toward descriptions of defined biological populations in (1) their present genetic structure, and (2) trends through time of gene frequencies, means, variances, and distributions of quantitative traits. Based on the above description, a quantitative trait may be defined as one influenced by the combined action of many genes, each with small effects relative to the observed variability of the trait. The analysis of such a continuously variable trait--height growth, for example--requires the use of statistically descriptive units such as means and variances. Such traits are said to be subject to the laws of quantitative inheritance. Since the effects of individual genes cannot be detected, gene frequencies and distributions cannot be determined.

Qualitative traits are categorized at the opposite end of the spectrum as those which can be uniquely described in terms of color, texture, size, migration distance, etc. Such traits are subject to the laws of particulate (rather than quantitative) inheritance because each variant can be related to a particular gene. This is also called Mendelian inheritance in honor of Gregor Mendel, who first described such inheritance on the basis of his classical experiments with garden peas. The utility of rare mutant genes in studies of population genetics of trees is based on the exactness of Mendelian inheritance, which permits prediction and interpretation of patterns and frequencies of mutants under specified hypothetical conditions.

ALLELIC BASIS OF MENDELIAN INHERITANCE

Chromosomes are the physical structures within the nucleus of a cell which contain most of the chemically encoded genetic information necessary for development and maintenance of a tree. Chromosomes occur in pairs; for example, pines have 12 pairs or a total of 24 chromosomes (Mirov 1967). As the term is commonly used, a gene is a genetic regulator which is located at a particular locus (place) on the chromosome. The chromosome consists of a long

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series of such loci arranged in definite linear order. Each member of a pair of chromosomes contains the same number and unique order of chemically encoded genes, each at its own locus. Thus, it is proper to say that the gene for a Mendelian trait is carried on a particular chromosome and that each ordinary cell of the tree will carry two genes for that trait.

The chemical arrangement (base pairing) of four simple organic molecules, called nucleic acids, determines the genetic information contained by a gene. This chemically coded genetic information is called an allele. The average gene contains 1,500 base pairs. By a change in base pairing, an allele can be altered chemically and changed from one code to another. Thus, the average gene could have 4^{1500} different alleles (Watson 1965). When the result of one such chemical change is seen in a tree, that result is called a mutation, i. e., a change in the chemical code of the gene forming a new allele which causes a distinct difference in the tree. The allele which causes the organism to appear "normal" is called the "wild type" and has the highest frequency in a natural population. Other alleles at the same locus are called mutants, and by definition are lower in frequency (rare) and do not produce the "normal" tree.

If a tree contains a wild type allele on one chromosome and a mutant type on the other, it will usually appear to be normal, just as though it had wild type alleles on both chromosomes. This property of the wild type allele is called complete dominance. Mutant alleles which are hidden by the wild type through dominance are called recessive alleles, and they appear in differing but predictable ratios (segregation ratios) when trees are crossed or selfed. In this way, mutants can be discovered, described, and evaluated for future use as genetic marker genes in forest genetics research (Franklin 1970).

DESCRIPTIONS OF MUTANTS

Morphological Description

Some of the most striking mutants in pine seedlings and trees are chlorophyll deficiencies of various types (Snyder et al. 1966, Franklin 1969). Color of the cotyledons in these mutants ranges from pure white, yellow, and yellow-green to pale green. Numerous other foliar variations are found including forms that change color through development (Kraus and Squillace 1964), repeat a color change annually, or show special coloration only under certain environments. Hypocotyls, primary and secondary needles, and even conelets and pollen catkins (Johnson and Critchfield 1974) may show color variants because of recessive alleles. Distinct variations in seedling and tree size and form, length of needles, and other morphological abnormalities have been described (Franklin 1970). There are so many possible alleles for each trait that the possibilities for variation are almost infinite.

Statistical Description

Statistical data such as segregation ratios and yields of filled seed after selfing and crossing, as well as frequency of the mutant alleles in the population, are necessary to assess the potential usefulness of a particular marker gene. A marker gene is one that has one or more mutant alleles which are suitable for interpreting patterns of gene transfer and assortment through a suxual generation within and between trees or within and between

populations.

Self-fertilization is one of the most efficient ways to discover and describe mutations. In loblolly pine, about one tree in four will show a distinct segregation pattern for at least one readily discernable recessive mutant allele causing abnormal coloration or form early in seedling development (Franklin 1969). The procedure is to self- and cross-pollinate several trees in a population of interest--for example, a seed orchard. When seed is collected and extracted, careful records must be kept on yields of filled and empty seeds. One effect of selfing is a large reduction in yield of filled seed in comparison with yields after crossing.

Seeds should then be sown in a greenhouse or other area of relatively moderate and controllable environment. Harsher environments such as a nursery result in fewer mutants being discovered and reliably described. This result occurs because of a combination of environmental effects on both the seedlings and the investigator. Careful observations and counts must be made on all seedlings, especially those which appear abnormal in any way. Once a mutant type can be confidently identified, frequency data can be used to estimate segregation ratios of mutant to normal types.

Under ideal circumstances, a tree containing one dominant wild type allele and one recessive mutant allele will yield 25 percent mutant offspring, 75 percent normal offspring when selfed. Since half of the pollen and half of the eggs contain the mutant allele, the probability that an individual has two normal alleles or two mutant alleles or one of each type is the product of the frequencies of those alleles (fig. 1). Chromosomal linkages frequently exist between mutant alleles under study and embryonic lethals

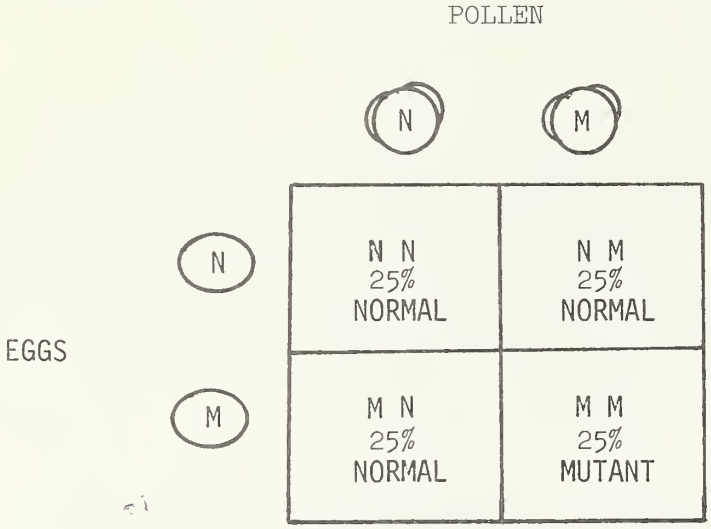

Figure 1.-- When a tree having one normal allele (N) and one mutant allele (M) is selfed, the result under ideal circumstances is equal frequencies of four types of offspring (genotypes), 75 percent of which are normal due to dominance of the normal allele and 25 percent of which are the mutant form.

(alleles which cause death of the embryo). In this case, segregation ratios may be distorted from the expected 3 to 1 ratio of normal to mutant types (Sorensen 1967). Therefore, the segregation ratio for each tree under selfing or crossing with another tree must be determined and confirmed before those alleles can be utilized as genetic markers. When chromosomal linkages cause simple Mendelian mutants to show up more frequently in a family than the expected 25 percent, this is an advantage because it increases the statistical precision of tests for a given number of offspring surveyed (Franklin 1974). On the other hand, if very few mutants appear in a family, prohibitively large numbers of seedlings must be surveyed, and that particular mutant allele is not a useful marker.

USES OF MARKER GENES

Although most mutants are deleterious, some have little noticeable effect on subsequent growth. This type mutant can be very advantageous if the abnormality can be observed early in seedling development. An example of such a mutant was described by Franklin (1969) as "green hypocotyl." As early as one day after germination, seedlings were distinguished as having either a normal (reddish-brown) or a mutant (green) hypocotyl. Beyond the cotylendonary stage, both types seemed to develop normally, including cone and seed production at ages 6 to 7 years. The significance of this type of mutant is that pure lines for the mutant type can be established and maintained by controlled crossing because all mutant types have only the mutant allele. An application of the green hypocotyl mutant for study of pollen contamination was made by Weyerhaeuser Company scientists, who established a small experimental seed orchard of loblolly pine consisting entirely of the green hypocotyl strain. When the orchard produces seed, all orchard-pollinated seedlings will have green hypocotyls, but pollen from the background pollen-contamination load will produce normal "wild type" seedlings. In this situation, the percentage of background contamination will be measured directly by the percentage of normal seedlings produced in the orchard. The fact that the mutant is very rare in the general population permits this direct approach.

Another example of the use of genetically marked pollen was reported by Franklin (1974). It involved a single slash pine tree (G-244) on the Austin Cary Forest, University of Florida, Gainesville. The objective of the study was to determine the effectiveness of the first pollen to reach a receptive strobilus versus the effectiveness of pollens reaching the strobilus 1, 2, 3, or 5 days later. The tree was known to produce progeny after selfing with relatively high and predictable frequencies of albino seedlings. The following controlled pollinations were repeated in two different years with the results being averaged:

1. Self-pollen from G-244.
2. Cross-pollen from a tree known not to carry the albino allele.
3. Mixture of 50 percent self and 50 percent cross pollen.
4. Self followed by cross pollen immediately and after 1-, 2-, 3-, and 5-day intervals.
5. Cross followed by self pollen immediately and after 1-, 2-, 3-, and 5-day intervals.

When self-pollen was applied first, its effectiveness was estimated directly as the percentage of selfing. When cross-pollen was applied first, its effectiveness was estimated as 100 minus the percentage of selfing, i. e., the percentage of crossing. The first pollen applied had an average effectiveness of 67 percent. Results were consistant with the hypothesis that the first pollen to reach a receptive strobilus was the most effective in accomplishing fertilization (table 1). Differences between self and cross pollens are consistent with a second hypothesis that when two or more fertilizations take place within the same ovule, cross-pollinated embryos usually outcompete self-pollinated embryos, resulting in higher yields of cross-fertilized seedlings. Thus, cross-pollen applied first averaged 78 percent, while self-pollen applied first averaged 56 percent effectiveness (table 1).

Table 1.-- Results of a controlled-pollination experiment showing that the first pollen received by receptive conelets was more effective in fertilization than pollens received later (from Franklin 1974).

Type of pollination	Number seedlings screened	Percent albinos	Estimated percent selfing	Percent effectiveness of 1st pollen	Mean percent effectiveness of 1st pollen
Self	264	32.2	100.0	---	---
Cross	456	0	0	---	---
50-50 Mixture	159	15.1	46.9	---	---
Self-cross 0[a/]	172	13.4	41.5	41.5	55.9
Cross-self 0	177	9.6	29.8	70.2	
Self-cross 1	285	17.5	54.5	54.5	66.1
Cross-self 1	223	7.2	22.3	77.7	
Self-cross 2	59	20.3	63.2	63.2	74.7
Cross-self 2	68	4.4	13.7	86.3	
Self-cross 3	86	17.4	54.2	54.2	63.5
Cross-self 3	103	8.7	27.1	72.9	
Self-cross 5	234	21.8	67.7	67.7	76.1
Cross-self 5	260	5.0	15.5	84.5	
Means: Self-cross				56.2	67.2
Cross-self				78.3	

a/ "Self-cross" denotes that pollen from the seed parent was applied first, whereas "cross-self" denotes that pollen from another tree was applied first. Numbers 0, 1, 2, 3, and 5 indicate days lapsed between application of 1st and 2nd pollens.

SUMMARY

These two examples of the application of mutant alleles as genetic markers to study genetic structure of populations differ distinctly in complexity as well as kind of information sought. There are many other possible applications, all of which rely on the same basic principles and considerations discussed here. The advantages of using genetic markers are:

1. No expensive or complex analytical equipment is needed.
2. Skills and procedures for crossing and selfing trees, and collecting, processing, and germinating seeds are readily available.
3. Useful mutants for markers are readily available in wild and selected populations.
4. Large populations of seedlings can be screened and scored easily.

Some disadvantages are:

1. Reliable physical and statistical descriptions of the mutant forms are necessary.
2. The relative rarity of most of these alleles limits some application possibilities.
3. Relatively low yields of seed from selfing often prohibit use of otherwise acceptable marker-bearing trees.
4. An 18-month wait is necessary to get control-pollinated seed.

Genetic mutants as marker genes may offer some especially attractive opportunities for research and assessment of patterns of pollen distribution in individual trees (Squillace and Kraus 1963), seed orchards (Franklin 1971, Kraus 1975), or under certain imposed conditions of control-pollination. Even certain inferences about wild stands can be made if population samples are large. The utility of these mutants must be evaluated against alternative methods before the best choice of methods can be made.

LITERATURE CITED

Franklin, E. C. 1969. Mutant forms found by self-pollination in loblolly pine. J. Hered. 60(6): 315-320.

Franklin, E. C. 1970. Survey of mutant forms and inbreeding depression in species of the family Pinaceae. USDA For. Serv. Res. Pap. SE-61, 21 p. Southeast. For. Exp. Sta., Asheville, N. C.

Franklin, E. C. 1971. Pollen management in southern seed orchards. Proc. 11th South. For. Tree Improv. Conf. 218-223.

Franklin, E. C. 1974. Pollination in slash pine: first come, first served. Proc. Seed Yield from Southern Pine Seed Orchards, p. 15-20. Ga. For. Res. Counc., Macon.

Johnson, L. C., and Critchfield, W. B. 1974. A white pollen variant of bristlecone pine. J. Hered. 65(2): 123.

Kraus, J. F. 1975. Estimates of selfed seedling production from a slash pine seed orchard based on gene markers. Proc. 13th South. For. Tree Improv. Conf.: 93-96.

Kraus, J. F., and Squillace, A. E. 1964. Inheritance of yellow oleoresin and virescent foliage in slash pine. Silvae Genet. 13(4): 114-116.

Mirov, N. T. 1967. The genus Pinus. 602 p. The Ronald Press Co., N. Y.

Snyder, E. B., Squillace, A. E. and Hamaker, J. M. 1966. Pigment inheritance in slash pine seedlings. Proc. 8th South. For. Tree Improv. Conf.: 77-85.

Sorensen, Frank. 1967. Linkage between marker genes and embryonic lethal factors may cause disturbed segregation ratios. Silvae Genet. 16(4): 132-134.

Squillace, A. E., and Kraus, J. F. 1963. The degree of natural selfing in slash pine as estimated from albino frequencies. Silva Genet. 12(2): 46-50.

Watson, J. D. 1965. Molecular biology of the gene. 494 p. W. A. Benjamin, Inc., N. Y.

ANALYSIS OF POPULATION STRUCTURE FROM ALLOZYME FREQUENCIES

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Abstract -- Selection strategies for tree improvement depend on population structure which will probably vary among species and even among regions within a species' range. Analysis of population structure using allozyme information can help in determining improvement strategy. Population structure can be described in terms of inbreeding, the grouping of genotypes within stands, and genetic distances among stands.

There was no evidence of inbreeding in stands of pitch pine based on analysis of 15 allozyme loci, nor was there evidence of familial groups within stands. However, there were differences among stands over distances of several kilometers. The implication for tree improvement strategy in pitch pine is to use comparison-tree methods of selection, and for provenance testing, to expend little effort on sampling different stands within regions.

INTRODUCTION

An important aspect of population structure is the subdivision of a species into smaller breeding units. In forest trees, adjacent individuals may have greater opportunities for mating than those separated by distance. Also, the closer two trees, the greater the likelihood they are related because of limited seed dispersal. Such situations could produce stands characterized by moderate levels of inbreeding and broken into family groups or neighborhoods. Alternatively, pollen and seed dispersal may be so great that genotypes are distributed at random within stands. Strategy and tactics of tree improvement should be chosen to take advantage of actual population structure, and in fact, tactics inappropriate to the population structure may limit improvement.

Allozymes are allelic variants of genes which code for enzymes and other proteins. Allozyme frequencies have been used by several authors to study population structure in forest trees (Feret, 1974; Sakai and Park, 1971; Sakai, Miyazaki and Matsuura, 1972; Rudin et al., 1974; Tigerstedt, 1973). In general, these workers found populations to be in close agreement with Hardy-Weinberg expectations, indicating that inbreeding was unlikely to be important. Nevertheless, differentiation between sub-populations was observed over distances as short as several hundred meters. Selection and genetic drift were suggested as factors responsible for the observed heterogeneity among populations.

As part of a continuing study of the genecology of pitch pine (Pinus rigida Mill.), we have used 15 allozyme loci to examine some aspects of population structure in natural stands. The analysis includes only populations from the central portion of the pitch pine range, and conclusions concerning the amount and distribution of variation may not apply to other ecological situations.

METHODS

Seed were collected from four populations of pitch pine in New Jersey, two

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from the dwarf Pine Plains (East Plains, N=153; West Plains, N=118) and two from the Pine Barrens (Lebanon State Forest, N=69; Helmetta, N=61). The Pine Plains and Lebanon populations are within 10 km of one another; the Helmetta population is located about 69 km to the north. All trees within a circle of approximately 50 m diameter and which were bearing cones (80 - 95% of all trees) were mapped and included in the sample. Handling of seed and conditions for horizontal starch gel electrophoresis are described in Guries and Ledig (1977). The loci analyzed included isocitrate dehydrogenase (IDH), fumarase (FUM), leucine aminopeptidase (LAP-1), acid phosphatase (ACP), aconitase (ACO), and two each of glutamate oxalate transaminase (GOT-1, GOT-2), malate dehydrogenase (MDH-1, MDH-2), phosphoglucomutase (PGM-1, PGM-2), glucose phosphate isomerase (GPI-1, GPI-2), and 6-phosphogluconate dehydrogenase (6-PGD-1, 6-PGD-2).

RESULTS

Within population comparisons

The frequency of the most common allele varied from 0.598 to 1.000 depending on gene locus and population (Table 1). Only three loci, GOT-2 and ACO in the West Plains population and MDH-1 in the Helmetta population, showed a significant deviation from the Hardy-Weinberg frequencies expected under random mating. Three deviations out of sixty cases could be expected by chance. However, it has often been noted that the Chi-square goodness-of-fit test lacks the statistical power to detect deviations due to inbreeding unless the sample sizes are very large or the inbreeding is pronounced (Ward and Sing, 1970; Smith, 1970). Inbreeding coefficients averaged over all loci were virtually zero for each stand (a maximum of 0.016).

Table 1.-- Frequency of the most common allele for 15 allozyme loci.

	Population			
Locus	East Plains	West Plains	Lebanon	Helmetta
MDH-1	.970	.997	.964	.959
MDH-2	.741	.765	.775	.721
IDH	.924	.850	.913	.959
FUM	.997	.996	.986	.984
PGM-1	.977	.962	.978	.992
PGM-2	.974	.987	.971	.934
GPI-1	.997	.991	1.000	1.000
GPI-2	.951	.974	.986	.967
6-PGD-1	.810	.716	.768	.623
6-PGD-2	.670	.754	.703	.598
LAP-1	.847	.912	.899	.951
GOT-1	.951	.924	.899	.893
GOT-2	.967	.934	.920	.951
ACP	.971	.962	.942	.926
ACO	.680	.725	.680	.598

Examination of the pattern of genotype distribution in the stands also provided no indication of inbreeding, i.e. clustering of genotypes. The distribution of MDH-2 genotypes in the Helmetta population (Fig. 1) is typical of the patterns observed for other loci in these stands. To statistically examine relationships among individuals with respect to their positions within the stand, we developed a genetic similarity value. Genetic similarity between individuals was calculated by averaging the number of alleles held in common over all loci. Each allele was weighted by its frequency. The measure is a genetic analogy to the "disagreement count" of Sakai and Miyazaki (1972) for scoring phenotypic banding patterns in peroxidase isozymes. Genetic similarity did not decrease with distance between individuals (Fig. 2). We suggest that the distribution of genotypes is essentially at random over areas as large as 2000 m^2 (0.2 hectares).

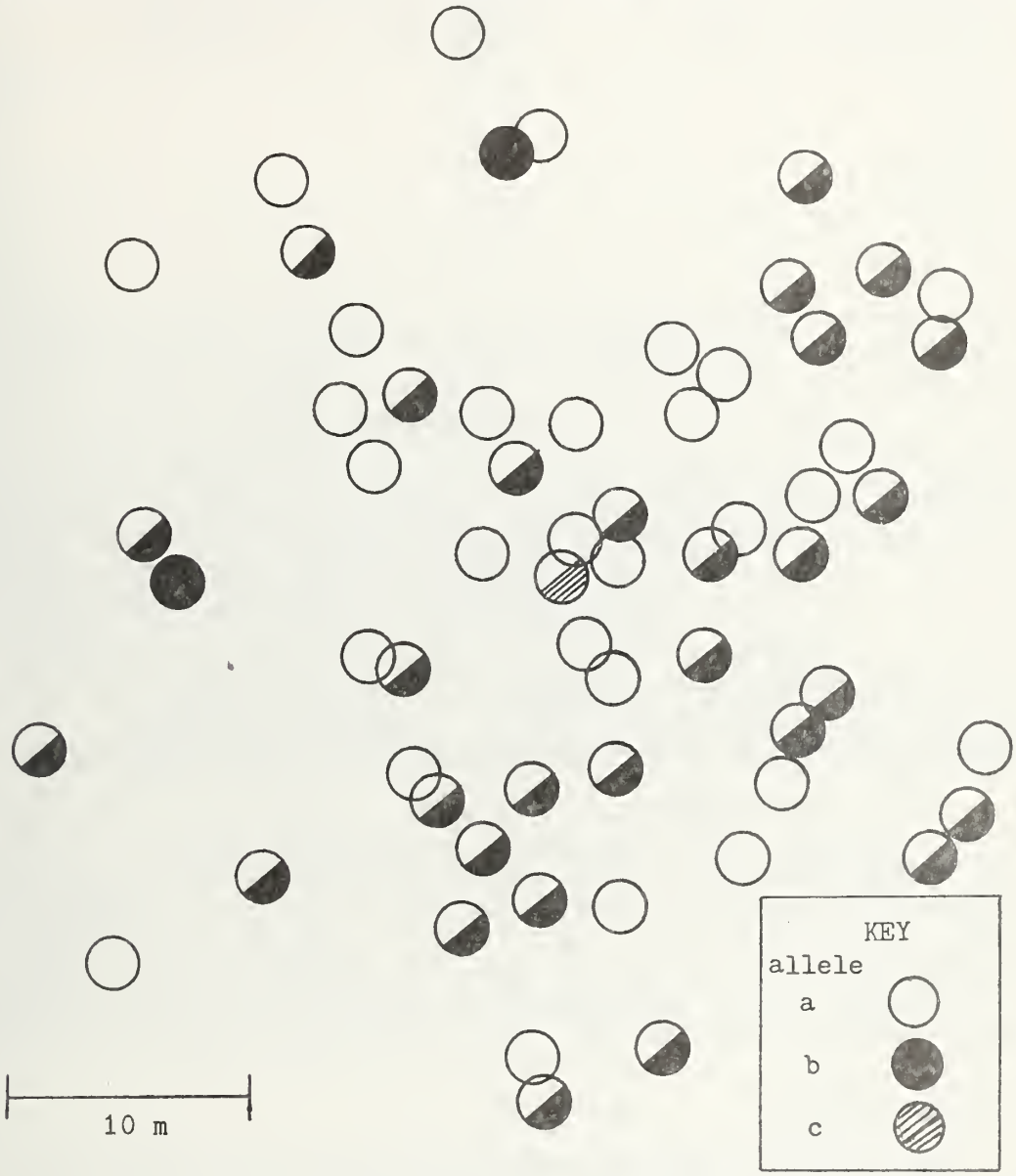

Figure 1. Spatial distribution of MDH-2 genotypes in the Helmetta population. Solid circles are homozygotes; split circles are heterozygotes.

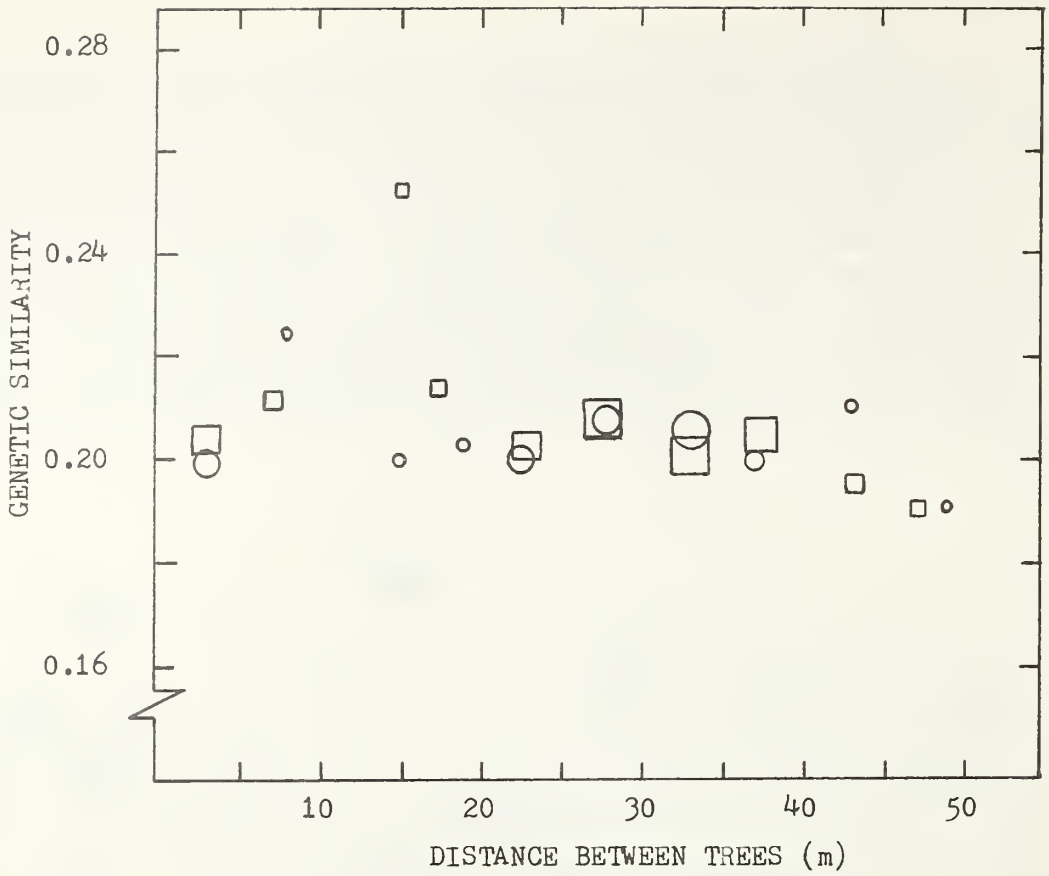

Figure 2. - Genetic similarity for trees 346 (O) and 190 (□) compared to 67 neighboring trees in relation to the distance between 346 or 190 and their neighbors. Points are means by 5 m intervals; size of symbol indicates the number of values included, from 1 to 15. Results for this pair of trees is typical for the Lebanon population, and regression for all 69 trees was approximately zero.

Between population analysis of gene frequency heterogeneity

Population subdivision is expected to result in a heterogeneous distribution of allele frequencies between subpopulations. Variations in allele frequencies can be tested by Chi-square contingency test (Workman and Niswander, 1970). There was significant heterogeneity of allele frequencies at most loci (Table 2), suggesting that the four populations of pitch pine were genetically differentiated. The variation in Chi-square values suggests that different loci are probably responding independently to the factors causing the heterogeneity.

Genetic distance

An alternative method of analyzing population structure is that of genetic distance. This technique measures the accumulated number of gene differences per locus among populations and expresses them in a simple index (Nei, 1972). If population differentiation is largely the result of isolation by distance, then spatial distance and genetic distance are expected to be positively related; i.e. as physical distance increases, genetic distance should also increase.

Table 2.-- Chi-square analysis of gene frequency heterogeneity among four pitch pine populations.

Locus	$X^2(3)$	p
MDH-1	14.90	$<.005$
MDH-2	4.36	$.10<p<.25$
IDH	14.84	$<.005$
FUM	7.51	$.05<p<.10$
PGM-1	3.19	$.25<p<.50$
PGM-2	24.90	$<.005$
GPI-1	5.62	$.10<p<.25$
GPI-2	8.76	$.01<p<.05$
6-PGD-1	38.01	$<.005$
6-PGD-2	13.04	$<.005$
LAP-1	19.54	$<.005$
GOT-1	15.06	$<.005$
GOT-2	5.70	$.10<p<.25$
ACP	5.20	$.10<p<.25$
ACO	22.26	$<.005$

Under an isolation by distance model, the Helmetta population, located some 69 km from the other three populations, would be expected to have the greatest genetic distance. However, the values for the Helmetta-Pine Plains comparisons were lower than those for the Lebanon-Pine Plains comparisons (Table 3), even though Lebanon is separated from the West Plains by a mere 5 km. The fact that large differences were not observed between Helmetta and the other populations suggests that isolation by distance may not be the major factor in population differentiation of pitch pine. While it is tempting to extrapolate to other wind-pollinated conifers, this conclusion must be accepted with caution owing to the small number of populations compared here.

Table 3.-- Estimates of genetic distance among four pitch pine populations. Values above diagonal are estimates of genetic distance, D; values below diagonal are estimates of the normalized identity of genes, I.

	Populations			
	East Plains	West Plains	Lebanon	Helmetta
East Plains		.0034	.0123	.0055
West Plains	.9966		.0102	.0065
Lebanon	.9877	.9899		.0147
Helmetta	.9945	.9935	.9854	

DISCUSSION

Pitch pine of the Pine Plains' and Pine Barrens' populations appear to be composed of individuals distributed randomly with respect to genotype. Vagile pollen and seed dispersal are the factors most likely to produce such a distribution over areas the size of those studied. The heterogeneity of gene fre-

quencies among populations indicates that differentiation has occurred over distances of several kilometers. Isolation by distance may be partially responsible for this differentiation, but additional factors such as environmental heterogeneity and genetic drift could also be involved. It is interesting to note that the dwarf populations of the Pine Plains are not radically different from the Pine Barrens' populations in terms of allozyme frequencies. These dwarf populations have originated and been maintained without extensive genic differentiation of the soluble enzymes investigated here.

Population structure should determine the pattern of sampling for provenance selection. If stands within regions are widely separated relative to pollen and seed dispersal distance, then adjacent stands may differentiate, and several stands will be required to adequately sample a region. Alternatively, large population size with wide outcrossing leads to predictable patterns of gene frequencies, and sampling need not be as intense.

Distribution of genotypes within stands also affects selection tactics and resulting gain. If adjacent individuals tend to be related, then rigidly applied comparison-tree methods of selection will provide less improvement than base-line selection (Ledig, 1974). Alternatively, if genotypes are randomly distributed without subdivision into local neighborhoods, then comparison-tree selection is favored because it provides a correction for environmental differences among select-tree candidates.

For pitch pine, inbreeding within clusters as small as 0.2 hectares is essentially non-existent, and genotypes are randomly distributed. Such a situation favors comparison-tree selection. While true for pitch pine, other species may differ in population structure as a result of ecological and genetic factors. For example, in white spruce (Picea glauca (Moench.) Voss.), and in Japanese arbor-vitae (Thujopsis dolabrata Sieb. & Zucc.) there was indication of substantial relationship among neighboring trees, which decreased as distance among trees increased (Coles and Fowler, 1976; Sakai and Miyazaki, 1972). In black spruce (Picea mariana (Mill.) BSP) the relationship among neighboring trees was slight except in small, isolated stands at the southern extreme of the range (Morgenstern, 1972). In Norway spruce (Picea abies (L.) Karst.) there was a completely random distribution of genotypes within stands (Tigerstedt, 1973). Indirect evidence suggests relationships among adjacent trees in some yellow pines (Snyder, 1969; pers. comm., 1973; Sittman and Tyson, 1971).

The relative amounts of genetic variation among individuals within stands and among stands or regions should also influence selection method. For allozymes there was little variation among stands within the same region, further suggesting the utility of the comparison-tree method of selection. The same conclusion was reached by comparison of provenance growth in nursery trials (Ledig et al., 1976). Genetic distances for allozyme loci did not increase appreciably with geographic separation, although admittedly results were based on limited comparisons. Differences among provenances in growth did increase clinally with respect to either latitude or climatic variables (Ledig et al., 1976). Thus, there must be some point at which variation among populations exceeds variation within, favoring some emphasis on provenance selection. Therefore, it seems reasonable to conclude that selection within regions should be sufficient to sample almost all the genetic variability available, although provenance selection might be a more efficient means of achieving some objectives. The impli-

cations for provenance versus within-provenance selection are not as clear as those for within-stand selection and further study is required.

The technique used here could be applied to any conifer, and would yield results in a short time. The benefit in planning tree improvement operations could be substantial, and studies using additional conifer species are currently under way.

ACKNOWLEDGEMENTS

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LITERATURE CITED

Coles, J. F., and D. P. Fowler. 1976. Inbreeding in neighboring trees in two white spruce populations. Silvae Genet. 25:29-35.

Feret, P. P. 1974. Genetic differences among three small stands of Pinus pungens. Theor. Appl. Genet. 44:173-177.

Guries, R. P., and F. T. Ledig. 1977. Inheritance of some polymorphic isoenzymes in pitch pine (Pinus rigida Mill.). Heredity: in press.

Ledig, F. T. 1974. An analysis of methods for the selection of trees from wild stands. For. Sci. 20:2-16.

Ledig, F. T., C. C. Lambeth, and D. I. H. Linzer. 1976. Nursery evaluation of a pitch pine provenance trial. In Proc. Twenty-third Northeast.For. Tree Improv. Conf., New Brunswick, NJ, pp. 93-108.

Morgenstern, E. K. 1972. Preliminary estimates of inbreeding in natural populations of black spruce, Picea mariana. Can. J. Genet. & Cytol. 14:443-446.

Nei, M. 1972. Genetic distance between populations. Am. Nat. 106:283-292.

Rudin, D., G. Eriksson, I. Ekberg, and M. Rasmuson. 1974. Studies of allele frequencies and inbreeding in Scots pine populations by the aid of the isozyme technique. Silvae Genet. 23:10-13.

Sakai, K.-I., and Y. Miyazaki. 1972. Genetic studies in natural populations of forest trees. II. Family analysis: a new method for quantitative genetics studies. Silvae Genet. 21:149-154.

Sakai, K.-I., and Y. Park. 1971. Genetic studies in natural populations of forest trees. III. Genetic differentiation within a forest of Cryptomeria japonica. Theor. Appl. Genet. 41:13-17.

Sakai, K.-I., Y. Miyazaki, and T. Matsuura. 1972. Genetic studies in natural populations of forest trees. I. Genetic variability on the enzymatic level in natural forests of Thujopsis dolabrata. Silvae Genet. 20:168-173.

Sittman, K., and H. Tyson. 1971. Estimates of inbreeding in Pinus banksiana. Can. J. Bot. 49:1241-1245.

Smith, C. A. B. 1970. A note on testing the Hardy-Weinberg law. Ann. Human Genet. 33:377-383.

Snyder, E. B. 1969. Parental selection versus half-sib family selection of longleaf pine. In Proc. Tenth So. Conf. For. Tree Improv., Houston, TX, pp. 84-88.

Tigerstedt, P. M. A. 1973. Studies on isozyme variation in marginal and central populations of Picea abies L. Hereditas 75:47-60.

Ward, R. H., and C. F. Sing. 1970. A consideration of the power of the χ^2 test to detect inbreeding effects in natural populations. Am. Nat. 104: 355-363.

Workman, P. L., and J. D. Niswander. 1970. Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. Am. J. Human Genet. 22:24-49.

SESSION V - MISCELLANEOUS

MODERATOR: WAYNE H. SMITH

GOT MACROGAMETOPHYTE ISOENZYMES OF VIRGINIA PINE [1/]

Peter P. Feret and Martha S. Witter[2/]

Abstract.--Analysis of populations of macrogametophytes from Pinus virginiana L. demonstrated genetic control at two loci for glutamate oxalo-acetate transaminase (GOT) isoenzymes. A comparison among four populations illustrated that natural stands and seed orchard progeny are not significantly different at the GOT locus A either in allelic composition or percentage of individuals heterozygous at the A locus.

The technique of protein electrophoresis permits the separation of multiple molecular forms of enzymes (Markert and Moller, 1959). Since different isoenzymes arise from different genes (Scandalios, 1974; Feret and Bergmann, 1976), isoenzyme analysis may be used to define genetic markers for studies relating to forest tree improvement and genetics.

In the Pinaceae, the genetics of isoenzyme inheritance may be investigated without extensive or intensive breeding programs. The nutritive tissue of pine seeds (female gametophyte tissue) originates from a single megaspore (Ferguson, 1904). The megaspore is the last remaining spore resulting from meiotic division of a megaspore mother-cell, the three other spores presumably disintegrating at random. Thus, analysis of female gametophyte isoenzyme variation among seeds collected from a single tree (genotype) will demonstrate segregation ratios if the tree is heterozygous. By analysis of a series of genotypes the inheritance mechanisms of isoenzyme phenotypes may be elucidated and genetic markers defined.

Reports may be found in the literature describing, for example, the use of isoenzyme genetic markers in studies relating to provenance variation (Bergmann, 1975; Rudin et al., 1974; Tigerstedt, 1973), seed lot and clone identification (Bergmann, 1972; Miyazaki and Sakai, 1969), and developmental genetics (Conkle, 1971; see also Conkle, this proceeding). Published reports relating an applied use of the technique for the solution of problems encountered in seed orchards and genetic management of seed orchard gene pools are scarce, if in existence at all. Thus, current studies by personnel of the U. S. Forest Service and State Universities are of extreme interest (Personal communication, R. Wier, North Carolina State Cooperative).

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[2/] Associate Professor of Forest Biology and Graduate Research Assistant, respectively. Department of Forestry and Forest Products, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061.

If inventories of isoenzyme genetic markers can be developed for the major southern pine species, it will be possible to accomplish the following:

1. Determine the amount of heterozygosity in seed orchards, seed orchard progeny, and natural stands.

2. Determine if gamete fertilization in seed orchards is random among clones.

3. Determine the percent of seed orchard progeny arising from selfing.

4. Identify clones or progeny for patent purposes, registration for seed certification, etc.

5. Monitor changes in gene frequencies as breeding programs move toward second and third generation populations to ensure maintenance of a proper "genetic base."

6. Provide quantitative measures of the effectiveness of "pollen management" procedures such as misting, etc.

7. Investigate the relationships between ubiquitous gene markers and economically important tree growth parameters.

Presented here are the results of an analysis of glutamate oxalo-acetate transaminase (GOT) isoenzymes in four populations of Virginia pine (Pinus virginiana L.). The analysis is presented as an example of how genetic markers can be used to compare gene and genotypic frequencies in domesticated and natural populations of Virginia pine.

MATERIALS AND METHODS

Virginia pine seeds were collected from four populations: the Virginia Division of Forestry seed orchard at Appomattox, Virginia, two natural stands, one near Critz, Virginia and the other at Blacksburg, Virginia, and a planted stand near Critz, Virginia, originating from seedlings obtained in 1971 as nursery-run stock from the Virginia Division of Forestry Augusta nursery. Following extraction from cones by air drying, seeds were stored at 4°C until used.

Female gametophyte tissue was extracted in 0.1 M, pH 8.0 Tris-HCl (.10 ml/seed), homogenized with a glass rod and centrifuged at 3500 XG for 10 minutes. Following centrifugation the supernatent was removed with a micropipette and layered into the electrophoretic apparatus.

Electrophoresis followed the methods of Davis (1964). Enzyme electrophoresis separations were made in 7.5% polyacrylamide gel slabs (.75 mm x 100 mm x 140 mm). Electrophoresis was conducted at 50 V and 7.5 ma for 100 minutes followed by 125 V and 12.5 ma for 130 minutes using a 0.09 M tris-glycine pH 8.9 reservoir buffer and a .2 M Tris-HCl pH 8.2 gel buffer. GOT isoenzymes were stained according to the procedures described by Schwartz, et al. (1963).

RESULTS

GOT Genetics

Using the techniques described above, eight GOT isoenzymes could be identified with a high degree of reliability (Fig. 1). Of the eight, those labeled $A3_1$ and $A3_2$ could not be reliably distinguished in some seed populations, thus $A3_1$ and $A3_2$ frequencies were combined and given the A3 designation.

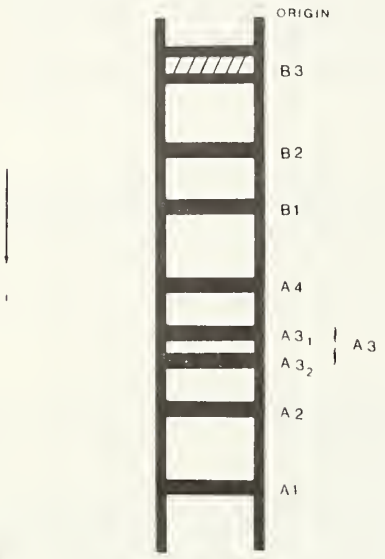

Figure 1.--Composite representation of the GOT isoenzymes in macrogametophyte tissue

Based upon the segregation patterns exhibited by seed from individual trees, there were found to be two loci: A and B. Locus A had four alleles (A1-A4, Fig. 1) and locus B, three alleles (B1-B3, Fig. 1). Chi square analyses of segregation ratios are presented in Table 1. Alleles A2 and A4 occurred in the samples analyzed here in intermediate frequencies, A3 less commonly, while A1 was found in one seed orchard tree. The B locus was essentially fixed for the B1 allele, except for one tree in the planted stand and one tree in the Critz natural stand. These two trees were heterozygous for B1/B2 and B1/B3 respectively.

Two trees in the sampled populations were heterozygous at both locus A and B; hence it was possible to test for linkage. The results of a Chi square test for linkage illustrated that linkage is not present (P = 0.05).

Table 1.--GOT segregation ratios for locus A and B derived from analysis of female gametophyte populations

Postulated seed tree genotype	No. trees analyzed	Sample size	Segregation ratio	χ^2	P
A2/A4	12	344	OBS: 169:175	0.10	0.7-0.8
			EXP: 172:172		
A2/A3	4	82	OBS: 46:36	1.22	0.2-0.3
			EXP: 41:41		
A1/A2	1	20	OBS: 10:10	0.00	> 0.9
			EXP: 10:10		
A3/A4	6	140	OBS: 73:67	0.26	0.5-0.7
			EXP: 70:70		
B1/B2	1	53	OBS: 25:28	0.17	0.5-0.7
			EXP: 26.5:26.5		
B1/B3	1	100	OBS: 52:48	0.16	0.5-0.7
			EXP: 50:50		

Population Comparisons

GOT allelic frequencies are listed in Table 2 by population. With the exception of the Blacksburg natural stand where sample size was a limiting factor, it can be observed that each population possessed a unique and relatively rare allele. A1 was unique to the seed orchard while B2 and B3 were unique to the Critz planted and natural stands, respectively. For the other allelic frequencies the four populations were not significantly different according to the arcsin transformation t test of Sokal and Rohlf (1961). Genotypic frequencies (Table 3) show a similar amount of homogeneity among the four populations analyzed. The only statistically significant differences were the frequency of A4/A4 genotypes in the Virginia Division of Forestry seed orchard compared with the Critz planted stand and the frequency of A2/A4 seed orchard genotypes compared with both Critz stands.

Table 2.--Frequency of the GOT alleles in four populations of Virginia pine.

Population	N[a/]	Locus A				Locus B		
		A1	A2	A3	A4	B1	B2	B3
Critz natural stand	20	0.00	0.50	0.02	0.48	0.98	0.00	0.02
Blacksburg natural stand	7	0.00	0.50	0.14	0.36	1.00	0.00	0.00
Critz planted stand	28	0.00	0.50	0.11	0.39	0.98	0.02	0.00
VDF seed orchard	25	0.02	0.34	0.06	0.58	1.00	0.00	0.00

[a/] Number of trees sampled

Table 3.--GOT genotype frequencies in four populations of Virginia pine

Population	N[a/]	Genotype									
		A2/A2	A3/A3	A4/A4	A2/A4	A2/A3	A3/A2	A1/A2	B1/B1	B1/B2	B1/B3
Critz natural stand	20	.20	.00	.15	.60	.00	.05	.00	.95	.00	.05
Blacksburg natural stand	7	.14	.00	.14	.43	.29	.00	.00	1.00	.00	.00
Critz planted stand	28	.21	.04	.11	.50	.07	.07	.00	.96	.04	.00
VDF seed orchard	25	.20	.00	.40	.24	.00	.12	.04	1.00	.00	.00

[a/] Number of trees sampled

A comparison is given in Table 4 of the four populations for the percentage of individuals found to be heterozygous at locus A. The data demonstrates that for GOT locus A the seed orchard was the most homozygous, heterozygosity occurring in only 40% of the trees. However, assuming panmixia, the levels of heterozygosity can be calculated for progeny of the populations from which seed was collected for this study. This was done using the gene frequencies given for locus A in Table 2. It was found that seed orchard progeny would be 54% heterozygous. This value is within the range found for the other three populations (Table 4).

Table 4.--Frequency of individuals heterozygous for GOT locus A in the four Virginia pine populations analyzed and (assuming panmixia) their progeny

Population	Frequency of heterozygous individuals	
	Population analyzed	Progeny of analyzed populations
Critz natural stand	.65	.52
Blacksburg natural stand	.71	.60
Critz planted stand	.63	.58
VDF seed orchard	.40	.54

DISCUSSION

The results of this study indicate that the technique of gel electrophoresis may be effectively utilized to study gene frequencies, genotypic frequencies and heterozygosity in southern pine populations. This can be done without breeding studies. For the GOT locus, allelic variability is approximately the same in the three population types studied here; these being natural stands, a stand derived from "nursery-run" stock and a seed orchard. Study of additional populations with larger sample sizes than those used here, coupled with additional gene loci and enzyme systems will permit application of the technique for the solution to the problem areas outlined above.

LITERATURE CITED

Bergmann, F. 1972. Experiments on possibilities for genetic certification of forest seed. IUFRO Genetics - SABRAO Joing Symposia, Tokyo, 1972.

Bergmann, F. 1975. Adaptive acid phosphatase polymorphism in conifer seeds. Silvae Genet. 24:175-177.

Conkle, M. T. 1971. Isozyme specificity during germination and early growth in knobcone pine. For. Sci. 17:494-498.

Davis, B. J. 1964. Disc electrophoresis. II. Methods and applications to human serum proteins. Annal. N. Y. Acad. Sci. 121:404-427.

Feret, P. P. and F. Bergmann. 1976. Gel electrophoresis of proteins and enzymes. IN: Modern Methods of Forest Genetics. Ed. J. P. Miksche. Springer Verlag, New York. pp. 49-77.

Ferguson, M. C. 1904. Contributions to the knowledge of the life history of Pinus with special reference to sporogenesis, the development of gametophytes and fertilization. Proc. Wash. Acad. Sci. 6:1-202.

Markert, C. L. and F. Moller. 1959. Multiple forms of enzymes: Tissue, ontogenetic, and species-specific patterns. Proc. Nat. Acad. Sci. 45: 753-763.

Miyazaki, Y. and K. Sakai. 1969. Use of zymography for identification of a clone in Cryptomeria japonica. J. Jap. For. Sco. 51:235-239.

Rudin, G., G. Eriksson, I. Ekberg, and M. Rasmuson. 1974. Studies of allele frequencies and inbreeding in Scots pine populations by the aid of the isozyme technique. Silvae Genet. 23:10-13.

Scandalios, J. G. 1974. Isozymes in development and differentiation. Ann. Rev. Pl. Physiol. 25:225-258.

Schwartz, M. R., J. S. Misselbaum, and O. Bodansky. 1963. Procedure for staining zones of activity of glutamic oxalo-acetic transaminase following electrophoresis with starch gel. Am. J. Clin. Pathol. 40:103-106.

Sokal, R. R. and F. J. Rohlf. 1961. Biometry. N. H. Freeman and Company, San Francisco. pp. 607-608.

Tigerstedt, P. M. A. 1973. Studies on isozyme variation in marginal and central populations of Picea abies. Hereditas 75:47-59.

A 16-YEAR PROVENANCE TEST OF LOBLOLLY PINE IN SOUTHERN ARKANSAS

Hoy C. Grigsby[1/]

Abstract.--Loblolly pine trees from seed sources throughout the range of the species were planted at two locations in southern Arkansas. Between ages 10 and 16 years, significant differences in growth altered the ranking (by volume) of the various seed sources. The correlation between closeness to coast of seed origin and fast growth evident at age 10, had begun to weaken at age 16, although it was still significant. The top-ranking three provenances in volume per tree and volume per plot were interior. The gain in volume of trees from interior sources over trees of coastal origin in this period was largely due to faster diameter growth. Considering both growth rate trends and susceptibility to fusiform rust, seed sources from the South Carolina Piedmont, Mississippi Central, and Mississippi Northern Coastal Plain were considered good choices for planting in southern Arkansas.

Additional keywords: *Pinus taeda*, seed source, *Cronartium fusiforme*.

Early tests of certain loblolly pine (*Pinus taeda* L.) seed sources have demonstrated substantial differences in the performance of various progenies in growth and resistance to fusiform rust, *Cronartium fusiforme* Hedg. and Hunt ex Cumm. (Wells and Wakeley 1966, Rink and Thor 1971, Goggans *et al.* 1972). These tests and others have shown that the prudent selection of seed sources can be an effective and economical way to increase yield.

The study described here was designed to test the performance in southern Arkansas of loblolly pine from seed sources throughout the range of the species. Survival, growth, and rust resistance of trees were observed and comparisons were made on data taken at ages 10 and 16 years.

METHODS

Seed samples were taken from the complete range of loblolly pine from Delaware to Florida and west to Texas (Figure 1). From a state, federal, or private agency at each of 32 locations, a 113-gram lot of nonselect seed was obtained. Planting stock was grown in an Arkansas Forestry Commission nursery and then outplanted in southwestern (Hempstead County) and southeastern (Cleveland County) Arkansas.

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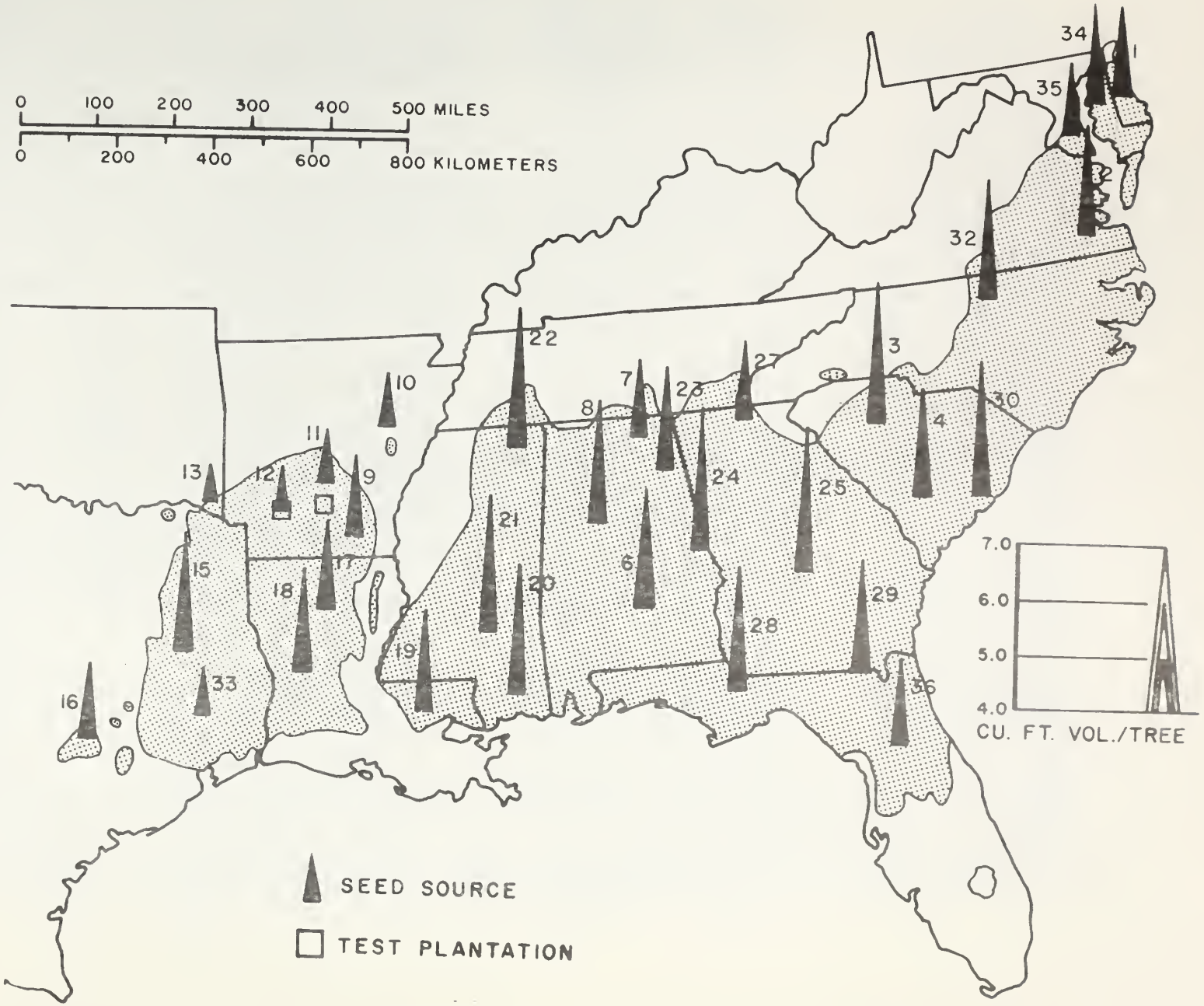

Figure 1.--Location and performance of seed sources from throughout the range of loblolly pine.

This report is based on eight plots of trees from each source--four in southwest Arkansas and four in southeast Arkansas. All plantings were arranged in randomized blocks. The southwestern plots contained 121 trees planted at a spacing of 6 x 6 feet. The 49 trees forming a square in the center of each plot were measured. The southeastern plots contained only 49 trees planted at a spacing of 8 x 8 feet, all of which were measured. Plots were contiguous and two rows of trees were planted on the outside boundary of the plantation to prevent border effects.

Field Data

About half the trees on each plot had been marked for removal at the time the 16-year data were taken. Trees to be removed were not measured. The largest and best trees were to be left and their height was measured to the nearest half-foot with telescoping poles, on alternate trees, until ten had been measured. Diameters at breast height were taken to the nearest tenth-inch on all trees to be left. Because thinning was in progress at the time of examination, survival and rust figures from data taken at age 10 were used. Trees having either stem or branch cankers were tallied as rust infected. A comparison with several unthinned plots indicated that both survival and rust percentages were relatively unchanged between ages 10 and 16.

Analyses

Survival, diameter, height, volume, and rust infection were examined by analyses of variance or covariance and multiple range tests. Volume per tree was computed with Schmitt and Bower's (1970) formula for young loblolly pines in plantations. Survival and rust data were transformed to arc sine $\sqrt{\text{percentage}}$. Differences among results from different sources were tested for statistical significance at the 0.05 level. Since radial stem growth is affected by stocking, diameter and volume per tree were adjusted by multiple covariance analysis for differences in survival. Average height was included as a second independent variable in the analysis to ensure that its impact on diameter and volume was not altered by the adjustment.

RESULTS AND DISCUSSION

Survival

Survival ranged from 64.4 to 93.4 percent, with a mean of 83.2 percent. The four provenances with the highest survival differed significantly from the one with the lowest (Table 1). In general, trees from low rainfall areas survived better than those from high rainfall areas, particularly those having mild coastal climates. No strong survival trends were apparent among sources from intermediate rainfall areas. Results from the Tennessee Valley (Zarger 1961), Georgia (Kraus 1967), and the Southwide Study (Collins 1964, Wells and Wakeley 1966), show that inland and western sources have also survived better than coastal or more eastern sources.

Table 1. Measurement data 16 years after outplanting in southern Arkansas. Sources ranked by volume.

Ident. Fig. 1	Origin of Seed	Vol. p/tree[1] (cu. ft.)[2]	Avg. DBH[1] (inches)	Avg. Ht. (feet)	Survival (%)	Rust (%)
24	Ala. East Central	6.65	7.70	49.3	81.0	19.9
25	Ga. Upper Coastal Plain	6.64	7.71	48.2	70.7	24.4
3	S.C. Piedmont	6.58	7.65	46.6	75.5	15.0
30	S.C. Lower Coastal Plain	6.47	7.62	50.0	77.7	11.4
21	Miss. Coastal Plain Central	6.45	7.51	48.5	88.5	9.0
22	Miss. Coastal Plain North	6.45	7.62	47.6	77.0	9.3
20	Miss. Coastal Plain South	6.30	7.45	47.8	80.8	11.6
28	Fla. Northwest	6.25	7.50	47.8	80.1	34.3
2	Va. Southeast	6.23	7.39	48.4	80.6	5.3
15	Tex. Coastal Plain	6.19	7.57	44.8	84.2	4.2
32	N.C. Piedmont	6.18	7.53	48.3	87.1	14.1
8	Ala. Piedmont	6.18	7.67	47.0	81.8	17.0
6	Ala. Coastal Plain	6.09	7.55	48.2	87.3	19.2
29	Fla. Northeast	6.00	7.45	48.2	70.6	30.4
4	S.C. Lower Coastal Plain	6.00	7.48	47.9	77.3	17.2
23	Ala. Mountain	5.92	7.43	47.5	88.2	11.8
18	La. Coastal Plain Central	5.86	7.37	45.5	85.9	6.0
19	La. Southeast	5.83	7.15	48.0	74.2	7.0
34	Md. Coastal Plain	5.83	7.25	48.7	93.4	4.1
1	Del. Coastal Plain	5.59	7.23	47.1	93.4	5.1
17	La.Coastal Plain North	5.58	7.30	44.5	83.5	6.9
36	Fla. Coastal Plain	5.52	7.23	45.7	64.4	6.1
9	Ark. Coastal Plain South	5.45	7.18	44.7	89.1	5.1
16	Tex. Lost Pines	5.43	7.28	42.1	86.2	4.9
7	Ala. Mountain	5.41	7.16	45.7	87.3	16.1
27	Ga. North	5.39	7.29	45.9	85.0	15.2
35	Md. Coastal Plain	5.32	7.23	47.0	82.3	5.5
11	Ark. Coastal Plain	4.96	7.06	43.0	89.8	6.6
10	Ark. Delta	4.95	7.08	43.8	84.2	4.8
33	Tex. Coastal Plain	4.86	6.78	45.2	80.1	2.5
12	Ark. Coastal Plain West	4.80	7.27	43.0	84.5	2.4
13	Okla. Coastal Plain	4.70	7.06	41.0	88.6	3.8

[1]Adjusted for survival in the presence of height by covariance analysis.

[2]Volume means not opposite the same line differ significantly by Duncan's test (P=0.05).

Fusiform Rust

Incidence of rust varied from 2.4 percent for local trees to 34.3 percent for those from northwest Florida (Table 1). The local trees and those from 13 other sources west of the Mississippi River had significantly less rust than trees from 11 eastern provenances.

Growth

Differences in the performance of trees from various sources were more obvious in diameter than in height growth. Diameters, however, responded noticeably to spacing. DBH decreased 0.2-inch and volume per tree decreased 0.3 cubic foot with each ten percent increase in survival. To correct this, DBH and volume per tree were adjusted downward if survival was less than the mean (83.2%) and upward if survival was greater than the mean. These adjustments changed the individual tree volume ranking of most sources from one to three places, and one source (No. 36) six places. They increased volume and diameter of western and interior sources, and decreased these attributes of coastal sources (Table 2).

Table 2.--Average diameter and volume per tree by regions before and after adjustment for survival.

Item	Unit	Western Sources[1/]	Eastern Sources Interior	Eastern Sources Coastal
Survival	Percent	85.4	82.7	79.5
Unadjusted diameter	Inches	7.16	7.49	7.43
Adjusted diameter	Inches	7.20	7.52	7.36
Unadjusted volume	Cu.ft/tree	5.22	6.11	6.07
Adjusted volume	Cu. ft/tree	5.28	6.18	5.94

1/ All western sources are interior.

At age 16, volume per tree varied from 6.65 to 4.70 ft^3 and some differences were significant (Table 1). Trees with the greatest volumes were from east central Alabama, west central Georgia, and the Piedmont of South Carolina. The trees of lowest volume were from western sources, including trees local to the planting site. The intermediate range in volume production was made up of trees from both coastal and interior sources. Coastal sources are considered to be those from within 50 miles of the Atlantic or Gulf coasts. Interior sources are from locations more than 50 miles inland.

The Livingston Parish, Louisiana source (No. 19), well known because it performed well in the Southwide Pine Seed Source Study (Wells 1969) in the southeast, dropped from eighth place in volume at age 10 to eighteenth place at age 16. This was not due to poor height growth, but rather to a slacking off of diameter growth. Also, the eastern Maryland source (No. 34) which ranked third in height, ranked nineteenth in volume because of poor diameter growth.

At age 10, there was a tendency for sources within about 50 miles of the Atlantic and Gulf coasts to excel in growth. The growth rate of most of these sources had slowed by age 16 and that of some of the more inland sources had accelerated. At age 10, coastal provenances comprised seven of the top ten sources in volume per tree (Grigsby 1973), while at age 16, the situation had reversed, and seven of the top ten sources were from more than 50 miles inland. Despite the tendency for coastal sources to decline, trees of one South Carolina coastal source (No. 30) have remained among the best performers in volume production. However, they have dropped from first to fourth place in six years. The current trend is for trees from inland sources between the coasts and the mountains to excel in southern Arkansas plantings, but later measurements will have to be taken to substantiate the trend.

The difference in individual tree volumes between loblolly from west and east of the Mississippi River is great. This difference became more marked between the tenth and sixteenth year. Average tree volumes at 16 years were 5.28 cubic feet for the western population and 6.06 cubic feet for the eastern. Eastern trees also grew more in height.

Trees from two general areas of the eastern part of the range have produced below average growth. One area is the mountains of northern Alabama and Georgia, and the other is the northernmost extremity of the range in Maryland and Delaware. This illustrates that sources of loblolly pine, even within the natural range of the species, are limited in their adjustment to new environments. The same northern Alabama and Georgia sources mentioned above have outperformed Coastal Plain sources when planted in Tennessee outside of the natural range of loblolly (Thor 1967, Zarger 1961).

DISCUSSION

In the present study and the Southwide Study, sources from northern Alabama and northern Georgia are growing slowly. Also, in both studies, loblolly pine from west of the Mississippi River is slower growing, rust resistant, and survives planting relatively well.

There are a few differences between the Southwide Study and the present study, such as the growth rate of the northeast Mississippi sources (fast in the present study and slow in the Southwide Study), and of the Maryland sources (slow in the present study and fast in the Southwide Study). However, growth rankings in the Southwide Study are made only on the basis of height (Wells 1969, Wells and Switzer 1975). If the same criteria were used in both studies, there would be even more agreement between them about seed source performance.

MANAGEMENT APPLICATIONS

Both the present study and the Arkansas plantation of the Southwide Study show that the Livingston Parish loblolly source (No. 19 in the present study) begins to lose vigor in the southern Arkansas environment after age 10. This indicates that the climate in this area, 50-75 miles from the northern limit of the natural range of loblolly, is too severe for trees from this Gulf Coast source. These results should not be viewed with alarm by those planters in the southeastern states who have elected to plant trees from this source in high rust areas in the Coastal Plain. However, it shows that caution should be used in planting seedlings from this source in the Piedmont and other areas near the interior boundary of the species' natural range.

Although trees of the four Arkansas sources had good records in survival and resistance to fusiform rust, they ranked near the bottom of the 32 sources in growth. Care should be taken when planting in Arkansas not to select seed sources from the high rust areas of Alabama, Georgia, and northern Florida.

LITERATURE CITED

Collins, A. G., III. 1964. Tenth-year results of loblolly pine seed source planting in Georgia. USDA For. Serv. Southeast. For. Exp. Stn. Res. Note SE-20. Asheville, N.C., 4 p.

Goggans, J. F., Lynch, K. D., Garin, G. I. 1972. Early results of a loblolly pine seed source study in Alabama. Auburn Univ. Agric. Exp. Stn. Circ. 194, 19 p.

Grigsby, H. C. 1973. South Carolina best of 36 loblolly pine seed sources for southern Arkansas. USDA For. Serv. South. For. Exp. Stn. Res. Pap. SO-89. New Orleans, La., 10 p.

Kraus, J. F. 1967. A study of racial variation in loblolly pine in Georgia--tenth-year results. In Ninth South. For. Tree Improv. Conf. Proc., p. 78-85.

Rink, G. and Thor, E. 1971. Results from ten loblolly pine provenance tests in Tennessee. In Eleventh South. For. Tree Improv. Conf. Proc., p. 158-164.

Schmitt, D. and Bower, D. 1970. Volume tables for young loblolly, slash, and longleaf pines in plantations in south Mississippi. USDA For. Serv. South. For. Exp. Stn. Res. Note SO-102. New Orleans, La., 6 p.

Thor, E. 1967. A ten-year-old loblolly pine seed source test in Tennessee. J. For. 65: 326-327.

Wells, O. O. 1969. Results of the Southwide Pine Seed Source Study through 1968-69. In Tenth South. For. Tree Improv. Conf. Proc., p. 117-129.

Wells, O. O. and Wakeley, P. C. 1966. Geographic variation in survival, growth and fusiform-rust infection of planted loblolly pine. For. Sci. Monogr. 11., 40 p.

Wells, O. O. and Switzer, G. L. 1975. Selecting populations of loblolly pine for rust resistance and fast growth. In Thirteenth South. For. Tree Improv. Conf. Proc., p. 37-44.

Zarger, T. G. 1961. Ten year results on a cooperative loblolly pine seed source test. In Sixth South. For. Tree Improv. Conf. Proc., p. 45-52.

GENOTYPE X MINERAL NUTRIENT INTERACTIONS WITH FUSIFORM RUST RESISTANCE IN SLASH PINE

Charles A. Hollis, Joel E. Smith and Robert A. Schmidt[1/]

Abstract.--The relationship of N, P and K availability to fusiform rust incidence in a resistant and a susceptible family of slash pine was examined in greenhouse pot cultures. Mean rust incidence for all treatments of the resistant family was 23.3% and was not significantly altered by application of N, P or K. A mean rust incidence of 53.8% was observed for all treatments of the susceptible family, with the addition of both P and K significantly increasing rust incidence. The P effect accounted for about 40% of the total variation in rust incidence due to treatment. Nutrient supply did not alter the relative resistance ranking of either family. Tissue nutrient concentrations in both families varied significantly with treatment. However, changes in rust incidence were not related to tissue nutrient content.

Additional keywords: Pinus elliottii, Cronartium fusiforme, forest diseases.

INTRODUCTION

Studies on mineral nutrient relationships with fusiform rust in southern pines have indicated that increased N and/or P supply generally resulted in increased rust incidence, especially if a nutrient deficiency existed prior to fertilization (Hollis et al., 1975; Rowan and Steinbeck, 1977). These studies were conducted on bulked seed lots from open pollinated pines and did not consider the host genotype as a source of variation in response to treatment.

Tree improvement programs have identified a substantial number of rust resistant families, some of which are likely to be planted in areas where fertilization practices are operational. This study was conducted to determine the effects of mineral nutrition on the infection of resistant and susceptible families of slash pine (Pinus elliottii Englem.) with fusiform rust (Cronartium fusiforme Hedgc.); and specifically to determine if resistant families would be predisposed by fertilization to fusiform rust.

METHODS AND MATERIALS

Seeds from two half-sib families of slash pine--one resistant and one susceptible--were obtained from the Florida Cooperative Tree Improvement Program. The seed was germinated in moist vermiculite and four seedlings were transplanted into 6 X 10 cm well drained plastic pots containing acid washed pure quartz sand. The seedlings were watered twice weekly with deionized water for four weeks prior to the initiation of nutrient solution application.

The effects of N, P and K fertilization and genotype were tested in a replicated 3 X 2 X 2 factorial experiment. Each family X nutrient treatment contained a total of 48 seedlings.

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The three elements were supplied in two concentrations, which were considered to be minimal and optimal concentrations for each. Nitrogen was supplied as: NH_4Cl/NH_4NO_3 (80 NH_4:20 NO_3 w/w) at 50 µg/ml or 100 µg/ml; phosphorus as NaH_2PO_4 at 5 µg/ml or 25 µg/ml; and, potassium as KCl at 50 µg/ml or 150 µg/ml. The basic nutrient solution applied to all seedlings contained: 0.5 µg/ml Mn as $MnSO_4$, 10 µg/ml Mg as $MgSO_4$, 7 µg/ml Ca as $Ca(NO_3)_2$, 2.5 µg/ml Fe as Fe-EDTA, 0.2 µg/ml B as H_3BO_3, 0.03 µg/ml Cu as $CuSO_4$, 0.03 µg/ml Zn as $ZnSO_4$, and 0.007 µg/ml Mo as $NaMoO_4$. Nutrient solutions were applied twice weekly in 50 ml aliquots. The day prior to treatment each pot was flushed with an excess of deionized water.

Five weeks following the initiation of nutrient applications, two seedlings were harvested from each pot for tissue analysis. The remaining seedlings which were of uniform height (about 12 cm) were inoculated with a spray suspension of basidiospores at about 50,000 spores per ml (Matthews and Rowan, 1972). Nutrient treatment was continued on a twice weekly basis.

Six months following inoculation, rust incidence was assessed and the seedlings were harvested. Healthy and diseased seedlings were separated and pooled within treatments and families for analysis. Foliar contents of N, P and K were determined as previously reported (Hollis et al., 1975).

Statistical significance was determined by analysis of variance and paired comparisons.

RESULTS AND DISCUSSION

The influence of mineral nutrition on fusiform rust incidence in susceptible and resistant families of slash pine is presented in Table 1. Mean rust incidence for the resistant and susceptible families was 23.3% and 53.8%, respectively. The susceptible family was infected to a significantly greater extent than the resistant family in all treatments and no change in the relative resistance ranking of either family was observed.

Table 1.--Percents of slash pine seedlings infected with fusiform rust as a function of family and N, P, K application

Element and Rate (PPM)	Resistant Family [a/]	Susceptible Family
	%	
N - 50	20.2 ± 4.1 A	51.8 ± 3.1 C
N - 100	26.4 ± 3.5 A	55.8 ± 5.1 C
P - 5	21.4 ± 3.8 A	47.4 ± 4.1 C
P - 25	25.2 ± 4.1 A	60.1 ± 3.0 E
K - 50	26.8 ± 3.7 A	49.9 ± 4.0 C
K - 150	19.9 ± 3.9 A	57.6 ± 4.1 D

[a/] Test means statistical significance: within family, same letter = NS; one letter difference, P = .05; two letters different, P = .01; between family means different at P ≥ .01.

Significant increases in rust incidence due to the application of high amounts of P and K were observed for the susceptible family; whereas, rust incidence in the resistant family was not significantly affected by nutrient application. No significant change in rust incidence in response to N addition was observed in either family. Previous reports (Rowan and Steinbeck, 1977; Schmidt et al., 1972) indicated that application of high amounts of N and/or P generally resulted in a significant increase in rust incidence. However, such studies used open pollinated families representing a broad spectrum of genotypes. Where specific families are used a more sensitive test of response to treatment should be expected due to the reduction in genetic variability.

Approximately 40% of the total variation in rust incidence in the susceptible family due to treatment was accounted for by the addition of P (Table 2). This substantiates previous reports that P availability has a profound influence on the incidence of rust in susceptible families (Schmidt et al., 1972; Hollis et al., 1975). The results of this study also indicate a probable cause for concern regarding the use of P fertilizers on planting stock, not selected for rust resistance, in areas where the potential for increasing rust incidence is great, i.e. where susceptible oaks are abundant.

Table 2.--Percentage of total variation in rust incidence accounted for by each significant source of variation. [a/]

Source of Variation	Resistant Family	Susceptible Family
N	N.S [b]	N.S
P	N.S	39.92 **
K	N.S	14.73 *
N X P	N.S	N.S
N X K	N.S	N.S
P X K	N.S	N.S
N X P X K	N.S	32.18 **
R^2 - Treatment	0.571 N.S	0.862 **

[a/] Percentage of total variation for each significant source calculated by dividing source mean square with sum of treatment mean squares. The quantity then being multiplied by 100.

[b/] Significance determined by ANOVA: N.S. = nonsignificant; *, P = 0.05; **, P = 0.01.

The addition of K produced contrasting results (Table 1). A significant increase in rust incidence in the susceptible family accounted for about 15% of the total variation due to treatment (Table 2). Such a response has not been previously reported for southern pines. In contrast a decrease in

rust incidence, albeit not significant, was observed in the resistant family due to K application. The wide range of variability in response to K between families expressed here points up the need for further efforts using genetic controls in studies of rust response to mineral nutrition.

Shoot nutrient content was examined at time of inoculation, and in healthy and diseased seedlings 6 months after inoculation. This was an attempt to determine whether shoot nutrient content at time of inoculation, or after symptom expression, had any direct relationship with the magnitude or direction or rust incidence response. Total shoot nutrient content at time of inoculation, for all three elements, significantly increased with rate of application for both families (Tables 3, 4, 5). However, only the addition of P and K to the susceptible family produced a significant increase in rust. This agreed with the findings of Rowan and Steinbeck (1977) that tissue nutrient content cannot be correlated with susceptibility to rust. The small differences between families in initial tissue concentration of the three elements also indicated that tissue susceptibility was not directly related to the absolute quantity of inorganic nutrients present.

Table 3.--Effect of N application on shoot nutrient content in seedlings of resistant and susceptible, half-sib, families of slash pine. [a/]

	Resistant Family		Susceptible Family	
	PPM Applied			
Seedling Condition [b/]	50	100	50	100
Initial	22.8±0.8 A [c/]	26.8±0.6 B	22.1±0.5 A	26.0±0.4 C
Healthy	7.6±0.4 AA	10.5±0.6 CA	9.9±0.4 AA	10.7±0.4 AA
Rust Infected	9.4±0.8 AB	10.6±1.3 AA	11.1±1.2 AA	8.4±0.5 BC

[a/] Nutrient content of shoot tissue expressed as µg N/g dry weight X 10^{-3}.

[b/] Seedlings within a family and treatment were pooled at time of harvest. Seedlings harvested 6 mos. after inoculation were segregated into healthy and rust infected groups.

[c/] Statistical significance: first letter is a comparison between rates within each family and condition, second letter is a comparison between conditions within each rate and family; same letter, NS; one letter different, P = .05; two letters different, P = 0.01. Significance determined by paired-t comparisons.

Table 4.--Effect of P application on shoot nutrient content in seedlings of resistant and susceptible, half-sib, families of slash pine. [a/]

Seedling Condition [b/]	Resistant Family		Susceptible Family	
	PPM Applied			
	5	25	5	25
Initial	18.5±0.4 A [c/]	34.8±1.0 A	19.6±0.5 A	38.6±1.0 C
Healthy	12.9±1.1 AA	26.2±1.4 CA	10.4±0.4 AA	24.6±1.6 CA
Rust Infected	10.5±0.4 AB	25.8±1.1 CA	13.2±1.4 AC	27.4±1.6 CB

[a/] Nutrient content of shoot tissue expressed as µg P/g dry weight X 10^{-2}.

[b/] Seedlings within a family and treatment were pooled at time of harvest. Seedlings harvested 6 mos. after inoculation were segregated into healthy and rust infected groups.

[c/] Statistical significance: first letter is a comparison between rates within each family and condition, second letter is a comparison between conditions within each rate and family; same letter, NS; one letter different, P = 0.5; two letters different, P = 0.01. Significance determined by paired-t comparisons.

Table 5.--Effect of K application on shoot nutrient content in seedlings of resistant and susceptible, half-sib, families of slash pine. [a/]

Seedling Condition [b/]	Resistant Family		Susceptible Family	
	PPM Applied			
	50	150	50	150
Initial	11.3±0.2 A [c/]	14.4±0.7 B	13.3±0.3 A	17.2±1.6 B
Healthy	9.5±0.5 AA	11.0±0.9 AA	11.4±0.8 AA	12.8±0.8 AA
Rust Infected	8.6±0.4 AA	10.8±0.7 CA	9.9±0.6 AB	12.9±0.5 CA

[a/] Nutrient content of shoot tissue expressed as µg K/g dry weight X 10^{-3}.

[b/] Seedlings within a family and treatment were pooled at time of harvest. Seedlings harvested 6 mos. after inoculation were segregated into healthy and rust infected groups.

[c/] Statistical significance: first letter is a comparison between rates within each family and condition, second letter is a comparison between conditions within each rate and family; same letter, NS; one letter different, P = .05; two letters different, P = 0.01. Significance determined by paired-t comparisons.

A comparison of the distribution of nutrients between healthy and diseased tissue should provide an insight into some of the nutritional requirements of obligate parasites, since tissue infected with an obligate parasite has often been considered to be a metabolic sink (Shaw, 1963). Such a comparison showed (Tables 3, 4, 5) that no pattern could be established for differences in elemental concentrations between healthy and diseased seedlings either within or among families or treatments. This contrasts the results of Martin (1972) that N and K concentrations were lower and P concentrations higher in blister rust infected tissue of western white pine. Perhaps a segregation of infected versus non-infected tissue within diseased seedlings would have provided more definite results.

The results of this study suggest that mineral nutrient applications do not predispose resistant families of slash pine to rust infection. Whether a broad range of nutrient application rates using genetic controls with varying degrees of resistance would produce substantially different results than this study remains to be tested in both the greenhouse and the field.

LITERATURE CITED

Hollis, C. A., W. H. Smith, R. A. Schmidt and W. L. Pritchett. 1975. Soil and tissue nutrients, soil drainage, fertilization and tree growth as related to fusiform rust incidence in slash pine. For. Sci. 21:141-148.

Martin, N. E. 1972. Influence of blister rust on inorganic solute concentrations in western white pine. Phytopathology 62:226-229.

Matthews, F. R. and S. J. Rowan. 1972. An improved method for large-scale inoculations of pine and oak with Cronartium fusiforme. Plant Dis. Reptr. 56:931-934.

Rowan, S. J. and K. Steinbeck. 1977. Seedling age and fertilization affect susceptibility of loblolly pine to fusiform rust. Phytopathology 67:242-246.

Schmidt, R. A., M. J. Foxe, C. A. Hollis and W. H. Smith. 1972. Effect of N, P, and K on the incidence of fusiform rust galls on greenhouse growth seedlings of slash pine. Phytopathology 62:788.

Shaw, M. 1963. The physiology and host-parasite relations of the rusts. Ann. Rev. Phytopathol. 1:259-294.

HOW MUCH GENETIC VARIANCE WILL BE REDUCED THROUGH CLONAL SELECTION?

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Abstract.--Clonal selection is defined here as vegetative propagation of selected clones. Clonal selection offers maximum genetic gain and maintenance of pure lines. Recently narrow genetic base has worried people working with clonal selection. The reduced genetic variance among selected clones can be expressed as $Vg' = Vg(1.0 - i\ h^2(i - c))$ where Vg is the original genetic variance before selection, i is the selection differential in standard deviation, h^2 is heritability and c is the truncation point in standard deviation.

Additional keywords: Phenotypic variance, pure line selection.

My definition of clonal selection is simply selection of clones for vegetative propagation. It should not be confused with producing seedlings from tested clones. The future clonal performance can be predicted from the test record because the genetically pure lines are perpetuated. But the seedling performance cannot be accurately predicted due to gene segregation and recombination.

The usual procedure for clonal selection is asexual reproduction of superior genotypes after a well designed, replicated clonal test. However, in the case of cloning plus-trees from phenotypic selection, or hybrid from hybridization, the original population may be considered as a clonal test with only one replicate per clone. The results from this study are still valid under this condition.

Clonal selection has been popular among horticulturists for years. Most of the fruit trees today belong to a few varieties of clones. In forestry, clonal selection has been successful for poplar (Schreiner 1959) and cottonwood (Mohn, Randall, and McKnight 1970). In the near future when we break thru the barrier for tissue culture; when silage silviculture becomes common practice; clonal selection will become more important.

Greatest genetic gain and uniformity can be obtained by selecting just one best clone. Unfortunately, narrow genetic base is usually associated with seriousness of disease problems and rigidness of adaptation requirements. In order to escape from these disadvantages, planting of clonal mixtures was recommended (Schreiner 1966).

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The question of how much genetic variability will be reduced by clonal selection is not easy to answer unless we have perfect information about the genotypes being selected, or when the number of clones is one. In the first case, we can compute genetic variances among individuals in the selected group as well as in the original population. In the second case the genetic variance is none for a single clone. If we have an estimate of clonal heritability and proportion of selection can we figure out the genetic variance of the selected clones? The answer is positive as indicated in this paper.

Computation of the Reduced Genetic Variance

The formula for computing the genetic variance among selected clones is as follows:

$$V = 1 - ih^2(i - c)$$

where V is the fraction of the original genetic variance
i is the selection differential in standard unit
h^2 is the clonal heritability
and c is the truncation point in standard unit

The proof of the formula is shown in the appendix at the end of this paper. However, I would like to illustrate here the procedure of computation and the implication of this formula.

After a clonal test, we can compute the clonal heritability as $h^2 = Vc/(Vc + Ve/n)$ as suggested by Burton and DeVane (1953). An easier way is to compute $h^2 = 1 - (1/F)$; where F is the F-value from analysis of variance table for the clonal test (Kung and Bey 1977). If the F value is non-significant the hypothesis of equal clonal mean should be accepted and no selection should be done. Then we need not be concerned about the reduction in genetic variance. On the other hand when the clonal means are significantly different then selection and heritability become meaningful.

Once we determine the level of culling or proportion of selection, the selection differential and cutoff point in standard deviation can be obtained from a table (Namkoong and Snyder 1969). For example, if we selected 10 percent, the selection differential (i) equals 1.7550 and the cutoff point (C) is 1.28155. Therefore, for $h^2 = .5$ the genetic variance is reduced from 1.0 to

$$1.0 - 1.7550 \times 0.5 \times (1.7550 - 1.28155) = 0.585.$$

Given the original genetic variance as 1.0 and heritability from .1 to 1.0 in steps of 0.1, the reduced genetic variance for various levels of selection proportions are shown in Table 1.

Table 1.--Fraction of genetic variance retained at various levels of heritability and selection proportion. The original genetic variance is 1.0.

Selection Proportion	Heritability									
	.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0
	Fraction of the Original Genetic Variance									
.90	.971	.942	.914	.885	.856	.827	.798	.770	.741	.712
.80	.958	.917	.875	.833	.791	.750	.708	.666	.625	.583
.70	.949	.899	.848	.797	.746	.696	.645	.594	.544	.493
.60	.942	.885	.824	.769	.711	.653	.596	.538	.480	.422
.50	.936	.873	.809	.745	.682	.618	.554	.491	.427	.363
.40	.931	.862	.794	.725	.656	.587	.518	.449	.381	.312
.30	.926	.853	.779	.706	.632	.559	.485	.412	.338	.265
.20	.922	.844	.766	.687	.609	.531	.453	.375	.297	.219
.10	.917	.834	.751	.668	.585	.501	.418	.335	.252	.169
.05	.914	.828	.741	.655	.570	.483	.397	.310	.224	.138
.01	.910	.819	.729	.639	.549	.458	.368	.277	.187	.097

A Practical Illustrative Example

A black walnut clonal test at Purdue University was reported to this Conference four years ago by Beineke and Masters (1973). Let me extract some of their data to illustrate the problem here.

Character	No. Clones	No. Grafts	Vc	Ve
Foliation date	50	224	24.866	2.310
DBH	17	68	0.062	0.184

Suppose that we would like to select half of the tested clones based on their performance in the nursery. How much genetic variance is there after vegetative propagation of these selected clones?

To use the formula or the table, we need to enter the value for clonal heritability. But first we have to know the average no. of grafts per clone. It can be seen that for foliation date the average no. of grafts per clone is n = 224/50 = 4.5; and for DBH it is n = 68/17 = 4. Then we can substitute needed data into the heritability formula as follows:

$$h^2 \text{ (foliation date)} = 24.866/(24.866 + (2.310/4.5)) = 0.98$$

$$h^2 \text{ (DBH)} = 0.062/(0.062 + (0.184/4)) = 0.57$$

When we select 50% of the population, the selection differential is .7979 and the cutoff point is at 0.0000 standard deviation. Given the original genetic variance as 1.0, the genetic variance for foliation date among selected clones is

$$1 - i\,h^2\,(i - c) = 1 - .7979 \times .98\,(.7979 - 0.0) = .38$$

Because the original clonal variance is 24.866, therefore, the actual genetic variance of foliation data among selected clones becomes 24.866 x .38 = 9.45.

By the same procedure, the actual genetic variance for DBH among selected clones is

$$0.062\,(1 - .7979 \times .57\,(.7979 - 0.0)) = .040$$

Discussion

Table 1 shows that small selection proportion and/or high heritability will cause greater reduction in genetic variance. When heritability equals 0.0, or when a trait is not genetically controlled, selection would cause no change in genetic variance. On the other hand, when heritability equals 1.0, or when a trait is controlled completely through genetics, the reduction in genetic variance equals the reduction of phenotypic variance. Therefore, the fraction of phenotypic variance maintained in the selection at various levels can be represented by the last column of Table 1. For example, if we want to know how much of the original phenotypic variance remains in the selection when 10% of the population is selected for a trait with $h^2 = .1$, we can see from Table 1 that the answer is .169, or about 17%.

Because the figures in the last column are the smallest ones among all columns, the reduction rate for phenotypic variance is greater than that for genetic variance. For example, when $h^2 = 0.1$ and selection proportion = .01, 91 percent of the original genetic variance is still retained in the selection while only 9.7 percent of the original phenotypic variance is represented among selected clones.

It comes to my surprise that the actual genetic variance may even become greater than the phenotypic variance in the selection. This happens when the proportion of selection is small and heritability is high. For example, assuming that the phenotypic variance of date of leaf fall as 100 and heritability as .90 then the original genetic variance would be 90. If we select 20% of clones, the phenotypic variance would be reduced to 100 x .219 = 21.9 while the genetic variance would be reduced to 90 x .297 = 26.7. However, it is true only in the selection and not in the next clonal propagation. As we can see in this example: the environmental variance is 100 - 90 = 10, the genetic variance of the 20% selection is 26.7. Assuming the environmental variance is the same for the next propagation, the phenotypic variance among the propagated, selected clones would become 26.7 + 10 = 30.7. Thus, the selected clones in the test plantation may seem to be

uniform. There may be more genetic variance in them than we can see on the surface. Certainly, if we propagate the selection again, they would become more different than they were at the time of selection.

As indicated earlier the formula can be used for vegetative propagation of phenotypic selection. The only change needed to be made here is the use of heritability for phenotypic selection rather than clonal heritability. Let us use data from Beineke and Masters again for illustration. The heritability for DBH given Vc = 0.062 and Ve = 0.184 is .062/(.0624 + .184) = .25. If we go out, select and graft 10% of black walnuts using diameter growth as our guide, the genetic variance for the four-year DBH would be 0.062 x .792 = 0.049. The value of .792 is interpolated between .834 and .751 which are the value at selection proportion = .10 and h^2 = .2 and .3 respectively in Table 1.

The narrow genetic base has worried many tree improvement workers. It is true that selection changes the variances and their relationship. The reduction in phenotypic variance can be easily seen. The reduction in genetic variance through clonal selection now can be computed. We can balance genetic variability with genetic gain to obtain an optimal selection level when we are working with vegetative reproduction. On the other hand, worry of narrow genetic base may be unfounded for sexual reproduction of truncated selection. During sexual reproduction the genetic variance depends on such things as gene frequencies, dominance, epistasis, linkage and the mating system. The genetic variance is changed by selection only slowly. The population is not likely to exhaust its genetic variance unless it is small, the number of loci is small, the selection is very intense and the mating scheme is very restrictive. None of above warnings can be applied to the present situation of seed orchard management. So we should just concentrate on maximizing genetic gain and not to worry about genetic variance among planted seedlings.

Literature Cited

Beineke, W. F. and Masters, C. J. 1973. Black walnut progeny and clonal tests at Purdue University. Southern Forest Tree Improve. Conf. Proc. 12th, 1973. pp. 233-242.

Burton, G. W. and DeVane, E. H. 1953. Estimating heritability in tall fescue (Festuca arundinacea) from replicated clonal material. Agron. Jour. 45:478-481.

Cohen, A. Clifford, Jr. 1959. Simplified estimates for the normal distribution when samples are singly censored or truncated. Technometrics 1. 217-237.

Falconer, D. S. 1972. Introduction to quantitative genetics. Ronald Press. New York, New York. 365 pp.

Kung, F. H. and Bey, C. F. 1977. Heritability construction for provenance and family selection. Manuscript prepared for the 13th Lake States Forest Tree Improvement Conference.

Mohn, C. A., Randall, W. K. and McKnight, J. S. 1970. Fourteen cottonwood clones selected for mid South timber production. South. For. Exp. Sta., U.S. For. Serv. Res. Pap. SO-62. 17 pp.

Namkoong, G. and Snyder, E. B. 1969. Accurate values for selection intensities. Silvae Genetica 18:172-173.

Schreiner, E. J. 1959. Production of poplar timber in Europe and its significance and application in the United States. U.S. Dept. of Agric. Handbook 150. 124 pp.

Schreiner, E. J. 1966. Maximum genetic improvement of forest trees through synthetic multiclonal hybrid varieties. Northeast. Forest Tree Improve. Conf. Proc., 13th, 1965. pp. 7-13.

Watterson, G. A. 1959. Linear estimation in censored samples from multivariate normal populations. Ann Math. Statist. 31, 814-24.

Appendix

Given a normal distribution with mean (μ), standard deviation (σ), truncation point (x), height of the ordinate at the point of truncation (z) and the proportion selected (p), we have standardized cut off point c and selection differential i as

$$c = (x - \mu)/\sigma$$

$$i = z/p \qquad \text{(Falconer 1972, p. 194)}$$

The variance among selected individuals after truncation, S^2, can be expressed as

$$S^2 = \sigma^2(1 - i(i - c)) \qquad \text{(Cohen 1959)}$$

Furthermore, let (X,Y) have a bivariate normal N (μx, μy, σx^2, σy^2, ρ) distribution, and let S^2 represent the variance in X after truncation. Then the variance Y after truncation can be expressed as

$$\text{Var } (y) = (1 - \rho^2)\ \sigma y^2 + \rho^2\ \sigma y^2 \sigma r{:}n^2 \qquad \text{(Watterson 1959)}$$

Replacing $\sigma r{:}n^2$ by $(1 - i(i - c))$ we have

$$\text{Var } (y) = (1 - \rho^2)\ \sigma y^2 + \rho^2 \sigma y^2\ (1 - i(i - c))$$

$$= \sigma y^2 - \rho^2 \sigma y^2\ (i(i - c))$$

Now, let us consider X as clonal phenotypic value and Y as clonal genetic value, and let us standardize the bivariate distribution as N (0, 0, 1, 1,ρ). Then we can see that ρ^2 is the heritability h^2. So we have

$$\text{Var } (y) = 1 - h^2(i(i - c))$$

$$= 1 - ih^2(i - c)$$

BLACK WALNUT POLLEN STORAGE AND GERMINATION

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Abstract.--Controlled pollination in forest trees often necessitates pollen storage for long periods. Early studies at Purdue University on black walnut pollen storage techniques demonstrated that refrigerator storage without dessication provides successful short-term storage (1 to 3 weeks), and later studies showed that storage in liquid nitrogen for several years was possible. Pollen germination of 24 clones revealed that germination of fresh pollen was 31.5% compared with 29.8% for pollen stored one year in liquid nitrogen. Germination fell to 23.1% and 13.3% respectively following two and three-year-storage. Pollen from the few clones available for germination tests after four,five, and six years storage in liquid nitrogen tested 58, 51, and 54% respectively. Pollen stored in liquid nitrogen for one year affected successful controlled pollinations with seed set and seed germination percentages as high as that obtained from fresh pollen.

INTRODUCTION

As programs for the genetic improvement of black walnut (Juglans nigra L.) advanced, it became necessary to investigate facets of control pollination in order to acquire the capability to maximize genetic gain. The paucity of information available on storage and viability of black walnut pollen led to this study.

Pollen storage is necessary primarily due to the wide variability associated with flower phenology. Unless pollen is successfully stored, certain specific crosses can not be made. For example, when pollen of one source matures a week in advance of pistillate receptivity, some means of short-term storage is needed in order to make that specific cross. If on the other hand, the situation is reversed, and the pistillate flower is receptive a week in advance of pollen maturation, the pollen would have to be stored for one year. Both of these situations occur in black walnut. In addition, maximizing genetic gain from seed orchards through mass supplemental pollination will require successful storage.

Storage of walnut pollen has been considered difficult, at least until recently. Griggs et al. (1971) stored Persian walnut (Julans regia L.) pollen for a year at -19°C with no adverse effects. Hall and Farmer (1971) proved

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that liquid nitrogen storage (-196°) of black walnut pollen was feasible, and they reported storage for several weeks without major loss of viability. Forbes (1974) used pollen stored in liquid nitrogen one year in making successful crosses in black walnut.

Viable pollen is necessary in a cross of any kind, and it becomes important to know the variability in pollen viability when considering a specific mating design. In addition, sources in the seed orchard with low pollen viability would require compensation for the lack of viable pollen from these sources. Wood (1934) reported the variability of pollen viability for Persian walnut to be 0 to 80 percent. Therefore, it was important to know if black walnut exhibited this same variability.

MATERIALS AND METHODS

Pollen storage

Attempts to store black walnut pollen at Purdue University began during the spring of 1970. Three different pollen sources were extracted and subjected to the following treatments.

1. Frozen in a standard household freezer at -15°C.
2. Stored in two refrigerators at 0 to 1°C, and 4 to 6°C.
3. Dried over silica gel in a refrigerated disiccator for 6, 12, and 24 hours, then frozen at -15°C.
4. Dried over silica gel in a refrigerated desiccator for 6, 12, and 24 hours, then removed from the desiccator but remained refrigerated at 0 to 1°C and 4 to 6°C.

After one week in storage, a random sample of 100 pollen grains per treatment combination were placed on germination medium and after 24 hours were observed under the light microscope to determine pollen germinability. If during microscopic examination, the pollen tube was seen, the pollen grain was considered germinated.

Liquid nitrogen storage at -196°C has been utilized for several difficult to store pollens including black walnut (Hall and Farmer 1971). From personal communication with R. Farmer, G. Hall, and A. Saki, valuable information was gained concerning the possibility of liquid nitrogen storage of black walnut pollen.

The liquid nitrogen storage procedure that we tested involved placing black walnut pollen in a 10 ml polycarbonate vial 16.2 X 79.4 mm, (Sorval Co., Newtown, Conn.) which was constructed to withstand the expansion and contraction associated with sudden changes in temperature involved with liquid nitrgen storage. The vials, half-filled with fresh pollen, were capped, then placed in specially designed liquid nitrogen storage unit (LD-17) manufactured by Union Carbide (Linde Division, Indianapolis, Indiana). This 17-liter unit required a monthly filling. Liquid nitrogen, if locally available is inexpensive.

To maintain pollen viability after removal from liquid nitrogen, the vials of pollen were immediately submerged in a 30° C water bath. This sudden rise in temperature is necessary to prevent moisture from crystalizing inside the pollen grain, which occurs between 0 and -20° C (Meyer and Anderson, 1952). At this point, the pollen was ready for control pollinations. If not used that day, it was stored in a standard refrigerator at approximately 4° C.

Pollen viability

Techniques and media described by Hall and Farmer (1971) were used to test pollen viability. The most desirable media for our purposes was found to be 200 gms. sucrose, 0.3 gms. boric acid and 6 gms. bacto agar in 1000 ml of water, heated until all agar flakes were dissolved, and the mixture cleared. Approximately 10 ml of the media were distributed to standard-size petri dishes, allowed to cool, then stored in a refrigerator.

In 1974, significant staminate flower production occurred in our clone bank at Martell Forest near West Lafayette, Indiana, and a study of pollen germination of 51 clones was undertaken. A random sample of mature catkins was collected in the afternoon, and allowed to dehisce overnight (18 to 20 hours) in a growth chamber maintained at 27° C and 40 percent relative humidity. The following morning a random sample of pollen was dusted on previously prepared medium. Twenty-three hours later, pollen germination was recorded. The germination percent was based on random sample of 250 pollen grains.

Considering the possibility that there could be variation associated with the time germination took place, a small study was set up to determine the period of maximum germination. Six clones were utilized in the study, and germination counts were made at 4, 10, and 23-hour intervals. Length of time fresh pollen remained viable at room temperature (23° C) was tested using these same clones by checking germination at 12 hour intervals until pollen no longer germinated on fresh medium.

Control pollination with stored pollen

The ultimate test of pollen viability after long-term storage in liquid nitrogen was the utilization of stored pollen in making control pollinations. Pollen stored for one year was utilized in making controlled crosses in 1972 and 1975. Pollination techniques and equipment were described by Beineke and Masters (1976).

RESULTS

Pollen storage and viability

Refrigerator storage, without desiccation, provided satisfactory short-term storage for one to three weeks (Table 1). Freezer storage and desiccator treatments were inconsistent and for the most part damaging. Black walnut pollen too moist for storage without desiccation is usually dead upon extraction. Immediately after extraction walnut pollen occasionally appears to be moist; i.e., it is dark yellow, does not flow freely, and tends to clump or cling together. Pollen in this condition is usually dead or has very low germination, and desiccation does not revive it. Moisture content of fresh pollen varies from 10 to 30 percent. Generally, moisture content above 15 to 20 percent shows the visual symptoms of moist pollen and will not germinate.

Table 1.-- Germination of 3 sources of black walnut pollen stored under 4 different refrigerator and freezer conditions.[1/]

Storage treatment[2/]	Percent pollen germination		
	Source 82	Source 102	Source 118
Fresh	18.4	48.1	50.5
	After one week		
Refrigerator- without desiccation (4 C)	16.5	19.6	36.2
D6R	4.6	4.1	6.8
D12R	8.7	6.4	19.0
D24R	7.9	24.2	26.0
Freezer-without desiccation (-15 C)	6.4	3.0	5.0
D6F	1.0	2.1	1.0
D12F	1.0	2.5	4.8
D24F	1.0	1.0	2.4
Room temperature (21-24 C)	0	0	0

[1/] Data from the two refrigerators were not significantly different and were averaged.

[2/] The symbolism refers to the hours pollen in a refrigerated desiccator (D), then taken out and stored in a refrigerator (R), or freezer (F).

Liquid nitrogen storage was successful for most clones for one year, and in fact, pollen germinability of some clones appeared to increase after storage (Table 2). This is probably due, however, to a difference in the condition of the germination medium used in different years. In terms of overall means, fresh pollen germinated 31.5 percent vs. 28.9 percent for pollen stored one year. After one year's storage, germination fell to 23.1 and 13.3 percent at 2 and 3-years, respectively. Germination of the few pollen sources available for testing at 4, 5, and 6 years, while much higher than expected, represent a small sample of sources that have already demonstrated their viability after at least one year's storage (Table 2). In 1974, the thorough fresh pollen germination study of all 51 clones available for testing produced an average of 35.7 percent germination which compares favorably to the 31.5 percent for clones stored in several different years. Pollen germination varied from 1 to 73 percent in the 51 clones in the 1974 test.

Pollen germination within clones can very radically from year to year. For example, fresh pollen from clone 31 germinated 10, 60, and 51 percent in 1971, 72, and 74, respectively (Table 2). However, some variation is probably due to media differences, counting techniques, timing of counting, and conditions of the catkins upon collection between years rather than true differences.

Table 2.--Germination percent of fresh and liquid nitrogen stored black walnut pollen.

Clone no. and yr. collected	Fresh pollen[1]	After liquid nitrogen storage					
		1 year	2 years	3 years	4 years	5 years	6 years
1-71	10	25					
4-71	0	30					
9-71	20		18				
21-71	15	25					
31-71	10	20					
31-72	60	20	0				
31-74	51	60	60				
34-71	50		1				
36-72	90		50			51	
36-74	45	25		25			
44-74	13	0					
48-71	15	30					
48-74	20	0					
49-71	10	10					
49-74	15	60	50	12			
55-71	40		1	10			
55-74	16	0					
71-71	10	5					
72-71	10		50				
82-71[2]	60	50		1	58		47
82-71	60	40		31			61
96-71	10	30					
102-71	50	50	0				
118-71	75	70	1	1			
Mean % Germ.	31.5	28.9	23.1	13.3	58.0	51.0	54.0

[1] Germination after the 16 to 24 hour extraction period.

[2] Pollen was taken from a syringe used for control-pollination in 1971 and has been thawed and replaced in storage 4 times.

As indicated earlier, some pollen sources stored in liquid nitrogen for more than one year were still viable. Even more remarkable, one vial taken from a syringe used for control pollinations in 1971, thawed, then replaced in liquid nitrogen stored 4 different years, germinated nearly as well in 1977 as it did fresh in 1971 (Table 2).

Liquid nitrogen storage of black walnut pollen was successful; however, germination of pollen placed in refrigerator storage after initial liquid nitrogen storage is considerably reduced after 24-hours in refrigeration. In a 7 clone test, pollen germination was 31 percent after one year in liquid nitrogen and the same pollen germinated only 13 percent after 24 hours in refrigerator storage.

Maximum pollen germination occurred in most clones four hours after being placed in the medium (Table 3). The drop in pollen germination percent over time, was due to pollen tube disintegration on the medium. Although clone 7 increased germination after four hours, additional experience since the data was collected, show that the time interval expressing maximum germination for most clones is in the six to eight hour range. If germination counts are delayed to the 23-hour period, disintegrated pollen tubes and fungus or bacterial growth often obscure live tubes and make counting difficult.

Table 3.-- Germination percent of 6 fresh black walnut pollen sources at 3 time intervals.

Clone No.	4 hours	10 hours	Net change (4-10 hours)	23 hours	Net change (10-23 hours)
86	24	20	-4	20	0
101	24	22	-2	21	-1
7	30	50	+20	42	-8
18	20	8	-12	10	+2
108	25	16	-9	17	+1
55	23	17	-6	16	-1
Mean	24.3	22.2	-2.2	21.0	-1.2

The final aspect of pollen viability information needed was the duration of viability at room temperature. In a small study using the clones in Table 3, pollen remained viable up to 96 hours at 23°C. The fact that pollen of all clones tested remained viable for at least 24 hours indicated there would be no problems with pollen viability during controlled pollinations.

Control pollination with stored pollen

Pollen stored in liquid nitrogen for one year affected successful controlled pollination in 1972 and 1975 (Table 4). Seed originating from stored pollen matured and developed normally, and seed set utilizing stored pollen was as good as that obtained from fresh pollen. In fact stored pollen source 31 produced double the seed set as compared to fresh pollen from source 31. Overall, stored pollen produced a slightly higher percentage of mature seed, 29 vs. 25 percent, than fresh pollen. Germination of seed which resulted from stored pollen was nearly the same as from fresh pollen (Table 4).

Table 4.--Success of control pollination using nitrogen stored pollen.

Year	Pollen source	No. flowers pollinated	No. mature seed set	Percent seed set	Percent seed germination
1972	102-stored 1 year	210	44	21.0	45.7
1972	Fresh pollen	599	143	23.9	47.2
1975	31-Stored 1 year	50	32	64.0	41.4
1975	31-Fresh	59	18	30.5	38.9
1975	Fresh pollen all sources	514	136	26.5	41.0
1972 & 1975	Stored	260	76	29.2	43.8
1972 & 1975	Fresh	1113	279	25.1	44.3

CONCLUSION

This long term study has shown that it is possible to store black walnut pollen for long periods and use it successfully in control pollination to obtain seedlings of known parentage. Problems similar to those encountered with black walnut pollen storage will occur with other hardwood species and hopefully some of the solutions that apply to black walnut will also remedy problems with other hardwood species.

LITERATURE CITED

Beineke, W. F. and C. J. Masters. 1976. Controlled pollination in black walnut. Proc. of the 10th Central States Forest Tree Impr. Conf., West Lafayette, IN. p. 66-72.

Forbes, D. C. 1974. Black walnut controlled-pollination techniques. Tree Planter's Notes 25(3):9-11.

Griggs, W. H., H. I. Forde, B. T. Iwakiri, and R. N. Assay. 1971. Effect of subfreezing temperature on the viability of Persian walnut pollen. Hort. Sci. 6(3):235-237.

Hall, G. C. and R. E. Farmer. 1971. In vitro germination of black walnut pollen. Canad. Jour. Bot. 49(6):799-802.

Meyer, B. S. and D. B. Anderson. 1952. Plant physiology. D. Van Norstrand Co., Princeton. 784p.

Wood, M. N. 1934. Pollination and blooming habits of the Persian walnut in California. U.S. Department Agr. Tech. Bull. 387, 56p.

QUALITY HARDWOOD SEEDLINGS REQUIRE EARLY MYCORRHIZAL DEVELOPMENT IN NURSERY BEDS

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Abstract.--Research during the past 3 years has revealed that many hardwood species require early endomycorrhizal infection in nursery beds to produce thrifty seedlings. If infection is delayed until late summer, as frequently occurs in production nurseries, seedlings of inferior grade are produced. In four different experiments with 18 half-sib progeny of sweetgum, root collar diameters of nonmycorrhizal seedlings averaged 0.17 cm and height averaged 5.0 cm, regardless of fertility regimes studied. Diameters and heights of mycorrhizal seedlings averaged 0.70 cm and 32.7 cm. These results indicate that seedling grade can be significantly improved by providing sufficient endomycorrhizal inoculum in nursery beds. The progeny from all our sweetgum selections have exhibited an obligate requirement for endomycorrhizal fungi, and all have produced good quality mycorrhizal seedlings. However, progeny from some mother trees have a greater percentage of larger-sized seedlings than progeny from others under a variety of experimental conditions. It may be feasible to develop a testing scheme to evaluate selected mother trees based on the morphological grade of their progeny in nursery beds under specified environmental conditions.

Additional keywords: Sweetgum, endomycorrhizal fungus, hardwood nursery practices.

Nurserymen find it hard to grow hardwood seedlings of consistently high quality for outplanting. This difficulty is a major obstacle to artificial establishment of many hardwoods. Research over the past 3 years, at the Forest Services' Mycorrhizal Institute in Athens, Georgia, suggests that a mycorrhizal deficiency in the nursery beds early in the growing season maybe a primary cause for the poor development of hardwood seedlings.

This conclusion is based on results from a large number of studies, many of which are still in progress. In this paper we make no attempt to describe our studies in detail or to prove our conclusions to some level of confidence. Our purpose here is to alert forest tree improvement workers to the possible importance of mycorrhizae for production of hardwood seedlings. Sweetgum is the primary example because our research is farthest advanced with that hardwood species. Despite their preliminary nature, we strongly emphasize findings of possible significance in tree selection and breeding.

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Infection delayed

Unlike pines and oaks which have ectomycorrhizal root symbionts, most hardwood species develop a symbiotic relationship with endomycorrhizal fungi. Spores of ectomycorrhizal fungi are readily disseminated by wind; those of endomycorrhizal fungi are disseminated primarily by soil water movement. Thus, while current nursery fumigation practices destroy the inoculum of both groups of fungi, ectomycorrhizal inoculum is restored to nursery beds much more quickly in the spring.

Reinfestation of nursery soil with endomycorrhizal fungi can occur in several ways. The root systems of hardwoods eventually grow below the zone of effective fumigation and come into contact with viable spores. Spores are moved into fumigated topsoil by earthworms and other microfauna. They are also moved in soil water movement during the growing season.

All these methods of bringing viable spores into contact with the developing root system have a major disadvantage: Infection does not occur until summer and there is insufficient time for seedlings to develop properly. The resulting seedlings are too large to plow under but too small to outplant.

The importance of endomycorrhizal fungi to hardwood seedling development was poorly understood until recently and research on it is still in the early stages. When seedlings were not developing well, no one thought of examining the roots under a microscope for the presence of endomycorrhizal symbionts. Nurserymen assumed that the problem was with moisture supply, fertility, or destructive root pathogens. The importance of endomycorrhizae for ion and water uptake by many hardwood species was not considered. Instead, fertility of nursery soils was increased--an approach that works with agronomic plants that lack their normal mycorrhizal associate. To obtain good growth of non-mycorrhizal crop plants up to 1000 ppm of phosphorus may have to be applied to the rooting media. Much work remains to be done before definite conclusions can be reached, but it appears that the same approach will not work with hardwood seedlings. Nursery beds may be overfertilized but few are underfertilized. The seedlings apparently must have endomycorrhizal associates.

The importance of endomycorrhizal fungi to hardwood seedlings growing in nursery beds was first observed by us with sweetgum (Liquidambar styraciflua L.) in 1973. Progeny from 10 selected mother trees were planted in beds that were improperly fumigated due to a sudden and prolonged drop in temperature during fumigation. By early July all seedlings were badly discolored and even weekly applications of liquid fertilizers throughout June and July elicited no visual growth responses. In late July and early August individual clumps of seedlings turned green and began to elongate. Laboratory examination of the root systems from these elongated seedlings showed them all to be mycorrhizal. The root systems from the still discolored and nonelongating seedlings proved to be nonmycorrhizal.

FORMAL STUDIES

We have worked with 10 hardwood species during the past 3 years. Sweetgum has been studied for 3 consecutive years, and only the results with this species will be considered in any detail here. The bulk of the initial nursery

work on the other 9 species was done during the 1976 growing season, and it is far too soon to be making conclusions about results.

Whenever possible, we planted half-sib progeny in our experiments. With sweetgum we recorded the location of the mother trees, thus some general conclusions about seed sources are possible.

Before seeding nursery beds we applied from 140 kg/ha to 1120 kg/ha of commercial 10-10-10 fertilizer, and we top dressed all seedlings including nonmycorrhizal controls with a total of 1680 kg/ha of NH_4NO_3 during the growing season. Calcium is important for hardwood root development and we have arbitrarily been standardizing this element in our microplots at 1120 kg/ha. All nursery soil is fumigated with MC-2 at the recommended rates to eliminate natural inoculum, destructive root pathogens and most weeds.

We maintain pure cultures of the endomycorrhizal fungi, Glomus mosseae and Glomus fasciculatus on sorghum roots in our laboratory. We also have a culture of mixed fungi obtained from a nursery bed on which sweetgum seedlings were growing in 1973. Several of the fungi in this mixture have not yet been identified. Infected sorghum roots are applied to treated plots. Soil leachates from the mycorrhizal culture are applied to control plots to standardize other soil microorganisms. Endomycorrhizal fungal spores are removed from these leachates with appropriate filters.

EARLY RESULTS

Since our primary purpose has been to improve nursery production of commercial seedlings, most of the seedlings have been destructively sampled. Relatively few have been outplanted. On the basis of past experience, however, it is reasonable to assume that large, thrifty nursery seedlings will survive and grow better than smaller ones.

All hardwood species studied thus far have benefited tremendously from the incorporation of mycorrhizal inoculum into the nursery beds at the time of sowing. Furthermore, sweetgum seedlings have demonstrated an obligate requirement for these root symbionts when grown in natural soils. Root systems of hardwoods are mycorrhizal in almost all natural situations.

We have 2 years of data (1974 and 1975) on total biomass on nonmycorrhizal and mycorrhizal sweetgum seedlings. The mycorrhizal sweetgum seedlings have been producing 70 to 80 times as much total dry weight as nonmycorrhizal ones. Comparable differences are apparent for most of the other hardwood species tested.

Table 1 shows differences in root collar diameter and total height among half-sib progeny of sweetgum seedlings after 1 growing season. Results are from four different studies conducted over several years. The results from a large sweetgum study completed in 1975 have already been submitted for publication. In this initial work we studied the response of sweetgum seedlings from eight different mother trees to different fertility regimes (140, 280, 560, and 1120 kg/ha of 10-10-10) with and without the endomycorrhizal fungus, Glomus mosseae.

Table 1.--Root collar diameter (RCD) and total heights (HT) of mycorrhizal and nonmycorrhizal sweetgum seedlings obtained from three experiments during 1975 and 1976.[1/]

Mother tree selection	Control		Glomus mosseae		Glomus fasciculatus		Natural inoculum	
	RCD cm	HT cm	RCD cm	HT cm	RCD cm	HT cm	RCD cm	HT cm
	1975 Experiment							
SG-74-2	.16	<5.0	.62	34.2				
SG-74-4	.16	<5.0	.57	34.1				
SG-74-5	.16	<5.0	.62	34.5				
SG-74-6	.16	<5.0	.73	40.5				
SG-74-7	.16	<5.0	.59	32.0				
SG-74-8	.16	<5.0	.70	35.8				
SG-74-9	.16	<5.0	.86	42.1				
SG-74-51	.16	<5.0	.66	32.5				
	1976 Experiment							
SG-SC-2	.19	3.8	.73	32.2	.80	36.5	.70	33.5
SG-SC-3	.17	3.6	.70	33.6	.93	41.2	.60	33.7
SG-SC-4	.18	5.2	.73	37.2	.92	43.9	.84	44.0
SG-74-7	.18	4.2	.75	30.4	.72	34.2	.88	34.8
SG-74-9	.22	5.7	.76	29.2	.85	30.2	.73	26.9
SG-75-2	.20	3.8	.60	25.8	.68	32.7	.48	22.9
SG-75-4	.16	3.5	.65	29.5	.70	29.4	.55	25.0
SG-75-6	.20	4.4	.66	31.1	.81	28.4	.69	29.7
SG-75-8	.17	4.1	.72	27.6	.71	29.0	.55	25.5

[1/] Seedling data not tabulated by individual fertilizer treatments. One can not compare a specific mother trees' progeny between years because of almost 40 days growing season between years due to differences in sowing dates.

In this 1975 study, the seedlings with mycorrhizae grew as well at the lowest rate as at the highest rate of fertilizer application. We also found that more of the progeny from upland selections grew to large sizes. In 1974, the progeny from a single upland selection were substantially larger than those from a bottomland selection.

These two observations indicate that progeny of some upland selections may glean a greater benefit from mycorrhizae than progeny of some bottomland selections. However, the upland sources may simply represent superior genotypes, via natural selection, that have been subjected to greater environmental stress on less fertile sites with significantly poorer soil moisture relationships. Thus, we do not mean to infer that all upland selections are better than all bottomland selections. Quite the contrary; we have tested some bottomland individuals whose seedlings were very good and we would readily include them among the better trees we have tested.

Genetic predisposition to endomycorrhizal infection has not been positively demonstrated to our knowledge within any forest tree species, but then no effort has been made to determine if it exists. Without outplanting data no strong

argument can be offered. The only information we have is from our nursery studies which suggest the possibility. Since we have studied at least 18 half-sib progeny of sweetgum, we will use this species to illustrate our point.

None of the progeny of sweetgum selections, regardless of fertility treatments, have developed much beyond the primary leaf stage without mycorrhizae. Progeny from all the parent trees have developed well regardless of fertility as long as they have mycorrhizae. This is dramatic proof of the obligate need of sweetgum for endomycorrhizae.

Some half-sib progeny have a higher percentage of large seedlings than other progeny. Thus, if an arbitrary size for superior seedlings is set 70 to 80 percent of the seedlings from one mother tree will meet the standard while only 30 to 40 percent of the seedlings from another mother tree may meet it. We realized that seedlings must be stratified by size and outplanted on many sites before the superiority of a given size class can be determined. However, based upon the distinctive results in our early tests, we feel it is possible and practical to develop nursery tests to identify mother trees which will produce seedlings of superior size with minimum fertility.

At this time, although the potential of our new mycorrhizal technology to the hardwood tree improvement programs in the Southeast is at best perhaps visionary, we feel its place in nursery management has been definitely established. Its implementation is imperative if we are to produce adequate numbers of plantable seedlings that are highly competitive in the field. Until recently there was little reason to presume that hardwood seedlings provided with adequate moisture and fertility in the nursery beds did not have a suitable environment in which to make optimum growth. Now, it has been demonstrated quite conclusively that hardwoods must have adequate levels of endomycorrhizal inoculum in the nursery beds early in the growing season it they are to attain the large size demanded by the forestry industries.

This new hardwood mycorrhizal technology will not need 10 or 20 years to be perfected. With a concerted effort and support by the forestry industries, this technology can be available in from 3 to 5 years. During this same time span, the potentials for incorporating mycorrhizal technology into the existing tree improvement programs can be easily explored. Foresters and the forestry industries are by nature conservative in accepting new practices. They should, however, carefully examine the potentials of this forestry related mycorrhizal technology instead of waiting for a decade to see what develops.

EVALUATION OF CLONE-NITROGEN INTERACTIONS IN POPULUS DELTOIDES

W. K. Randall and B. G. Blackmon [1/]

Abstract.--Cottonwood (Populus deltoides Bartr.) growth response to three levels of fertilizer nitrogen was nonsignificant for nine cottonwood clones (three good, three average, three poor growers) evaluated on a Convent soil in a greenhouse study. Clone differences were significant for some growth characters and foliar nutrient levels. Clone X nitrogen interaction was nonsignificant. For 8 of 15 characters measured, the good clone class was significantly better than the poor clone class. Only for leaf weight and foliar nitrogen did nitrogen variance exceed clonal variance. Results of this greenhouse study do not support known nitrogen response from the same soil under field conditions. Thus greenhouse results must be viewed with caution and field tests undertaken prior to nutrient and clonal recommendations.

Additional keywords: Cottonwood, fertilization, foliar analysis.

Cottonwood (Populus deltoides Bartr.) growers are interested in the possibilities of using fertilizer to speed tree growth. Plantations composed of genetically mixed material have responded markedly to nitrogen fertilization (Blackmon 1973, Blackmon and White 1972). But it is possible that some clones will respond favorably to additions of nitrogen fertilizer while others will not. Indeed, there is evidence to indicate that such a varied response is true. For example, Curlin (1967) reported a clone X fertilizer interaction for young cottonwood. Randall and Mohn (1969) found a significant clone-site interaction in the Mississippi River floodplain, and more recently Baker and Randall (1974) found foliar nitrogen to be associated primarily with a clone X soil interaction. The present study was designed to investigate a possible genotype X nitrogen interaction in eastern cottonwood.

METHODS

Soil used was a Convent silt loam (Aeric Fluvaquent) collected from the surface foot in an old field on the Fitler Managed Forest (west-central Mississippi). This soil is known to be low in nitrogen, having only 0.058% total N, although some Convent soils which are not deficient contain up to twice this concentration of N. After air drying, the soil was shredded to

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pass a 1/4-inch screen, thoroughly mixed, and 22 pounds were placed in 3-gallon earthenware glazed crocks.

Nine clones were selected on the basis of prior field performance to represent three clone classes as follows: good--Stoneville 66, 240, and 244; average--Alton 1, Stoneville 213 and 153; poor--Rosedale 8, Stoneville 29 and 171.

Terminal, greenwood cuttings 4 inches long were taken from nursery stock in mid-May. The cuttings were placed in a 2-inch-square peat pot filled with a 2:1 mixture of sand and perlite and placed in a mist chamber for approximately 2 weeks, by which time roots were about 1 inch in length. Two cuttings were then transplanted to each of the 3-gallon crocks. As soon as the plants became established (about 2 weeks), the poorer plant was removed from each crock. Three rates of nitrogen equivalent to 0, 150, and 300 pounds per acre were applied by spreading ammonium nitrate onto the soil surface, stirring the soil surface with a small garden spade, and watering immediately. Moisture content was maintained within the range of 50 to 80 percent of field capacity.

Plant heights and groundline diameters were measured immediately before nitrogen application. Ten weeks later, final measurements were taken which consisted of height, groundline diameter, number of leaves, oven-dry weight of leaves, stem, branches, roots, total stem, and total plant. Leaves were analyzed for nitrogen by the micro-Kjeldahl method. Phosphorus was determined by absorption spectrophotometry, and potassium, calcium, and magnesium concentrations were determined by atomic absorption spectrophotometry.

Experimental design was a randomized complete block with factorial arrangement within blocks, resulting in 27 treatments replicated 3 times. Clone and nitrogen were considered as fixed factors. All statistical tests were made at the 0.05 level. Means were compared by Duncan's new multiple range test.

RESULTS

Clones differed significantly for all characters except leaf weight and foliar Mg and P concentrations. For 8 of the 15 characters, means of the good and average clone classes were the same. The good and poor clone classes were significantly different for eight of the characters (Table 1).

The good clone class was taller, had more leaves, greater stem and branch weight, greater root weight, greater total stem weight, and greater total plant weight than the poor clone class. Good clones had lower foliar Ca and N concentrations than did the poor clones. The average clone class was generally not significantly different from either the good or poor class.

Before nitrogen was applied, there were no height or diameter differences among fertility level plots. Final measurements indicated no positive diameter or height response to N, but the number of leaves increased and root weight decreased with increasing rates of N. The total aboveground portion of the plant and the total plant remained unchanged. Foliar nutrient levels of N, P, K, Ca, and Mg were increased by the addition of N (Table 1).

Clonal variance generally far exceeded variance due to nitrogen levels. Only for foliar N and leaf weight was the fertilizer variance larger than clonal variance. The clone X nitrogen interaction was nonsignificant for all characters, and in most cases the amount of variance was extremely small.

Table 1.--Means for three levels of nitrogen fertilizer and three clone classes

Character	Fertilizer N level (lbs/ac)			Clone class		
	0	150	300	Good	Average	Poor
Initial height (cm)	10.7a[1/]	10.9a	10.5a	10.8ab	11.8a	9.4 b
Initial diameter (mm)	5.4a	5.0a	5.2a	5.4a	5.3a	5.0a
Final height (cm)	77.2a	77.8a	72.1 b	78.9a	80.5a	68.0 b
Final diameter (mm)	7.8a	7.8a	7.6a	8.0a	8.2a	7.0a
Number of leaves (no)	16.3 b	18.2ab	19.1a	19.4a	16.8 b	17.5 b
Leaf weight (gr)	8.4 b	9.4a	8.6ab	9.6a	9.2a	7.4a
Stem & branch weight (gr)	6.4a	6.6a	6.0a	6.6a	7.3a	5.2 b
Root weight (gr)	6.4a	5.5 b	5.2 b	6.4a	5.5 b	5.1 b
Total stem weight (gr)	15.9a	17.5a	16.9a	17.4a	17.7a	15.2 b
Total plant weight (gr)	22.3a	22.9a	22.1a	23.8a	23.3a	20.3 b
Foliar nitrogen (percent)	2.14a	2.85 b	2.99 c	2.56 b	2.73a	2.69a
Foliar phosphorus (percent)	0.18a	0.20 b	0.20 b	0.20a	0.20a	0.19a
Foliar potassium (percent)	2.40a	2.56 b	2.55 b	2.41 b	2.63a	2.47 b
Foliar calcium (percent)	1.65a	1.84 b	1.93 c	1.75 b	1.82ab	1.85a
Foliar magnesium (percent)	0.49a	0.54 b	0.54 b	0.53a	0.51a	0.53a

[1/] Character means with the same letter are not significantly different at the 0.05 level.

DISCUSSION

This study produced two major surprises: one was the lack of a growth response to fertilizer N, and the other was that the clone X nitrogen interaction was nonsignificant. Even though field studies of the N-deficient Convent soil have shown substantial growth increments with the addition of N (Blackmon 1973), a lack of response in the greenhouse perhaps does not conflict with what is known about this soil. Blackmon (1974) stated that cottonwood trees are unlikely to respond to fertilization until they fully utilize the site and are competing with each other for nutrients. The small amount of

available nitrogen was probably sufficient to allow for 12 weeks of thrifty growth. A foliar N level of 2 percent was determined as minimum for good cottonwood growth (Blackmon and White 1972). In our study, even the leaves of the unfertilized plants had N concentrations above the 2-percent level. Foliar N levels plus the general plant vigor indicated that there were no N deficiencies.

Curlin's (1967) work provided ample evidence that cottonwood clones do interact with levels of N. More recently, Baker and Randall (1974) found a strong clone X soil interaction. The former reported on nursery plants for two growing seasons and the latter with 4-year-old trees on two contrasting sites.

The results reported here indicate that caution should be used in interpreting greenhouse results since they may be poorly correlated with field responses. Curlin (1967) concluded that the geneticist must be careful to carry out individual selection in the nutritional environment in which the tree will ultimately grow. Baker and Randall (1974) concluded that fertility tests should be conducted on more than one soil.

Future greenhouse tests to screen clones for nutrient-clone interactions should employ a growth medium known with certainty to be nutrient-deficient. One possibility would have been to use sand culture and nutrient solutions as employed by Steinbeck (1971) in his work with genotype-nutrient interactions in sycamore. Another way would have been to mix soil with an inert substance such as sand. The growth medium would probably have been nitrogen-deficient even for young plants. Then, nitrogen responses and perhaps nitrogen-clone interactions would likely have been observed.

LITERATURE CITED

Baker, James B., and Randall, W. K. 1974. Foliar nitrogen and potassium variation in cottonwood as affected by genetic and site factors. Ninth Cent. States For. Tree Improv. Conf. Proc. 1974: 106-111.

Blackmon, B. G. 1973. Response of eastern cottonwood plantations to fertilization: Rates of nitrogen. Agron. Abstr., p. 137-138.

Blackmon, B. G. 1974. Hardwood fertilization: Research progress in the Midsouth. Southeast. Area Nurserymen's Conf. Proc. 1974: 51-58. USDA For. Serv., Southeast. Area, State and Priv. For., Atlanta, Ga.

Blackmon, B. G., and White, E. H. 1972. Nitrogen fertilization increases cottonwood growth on old-field soil. USDA For. Serv. Res. Note SO-143, 5 p., South. For. Exp. Stn., New Orleans, La.

Curlin, James W. 1967. Clonal differences in yield response of Populus deltoides to nitrogen fertilization. Soil Sci. Soc. Am. J. Proc. 31(2): 276-280.

Randall, W. K., and Mohn, C. A. 1969. Clone-site interactions of eastern cottonwood. Tenth South. For. Tree Improv. Conf. Proc., p. 89-91.

Steinbeck, K. 1971. Growth responses of clonal lines of American sycamore grown under different intensities of nutrition. Can. J. Bot. 49: 353-358.

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