24th Biennial

Southern Forest Tree Improvement Conference

Orlando, Florida, USA • June 9-12, 1997

PROCEEDINGS

Sponsored by

The Southern Forest Tree Improvement Committee

Hosted by

UNIVERSITY OF FLORIDA

Institute of Food and Agricultural Services

School of Forest Resources and Conservation

Proceedings of the 24th Biennial Southern Forest Tree Improvement Conference
Compiled by Tim White, Dudley Huber and Greg Powell

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Foreword

The University of Florida, School of Forest Resources and Conservation was proud to sponsor the 24th Biennial Southern Forest Tree Improvement Conference (SFTIC), the North American Quantitative Forest Genetics Group (NAQFGG) and the Technical Association of Pulp and Paper Industries (TAPPI) meetings. These meetings took place June 9-12, 1997 in Orlando, Florida at the Holiday Inn International Drive Resort.

There were a total of 8 presentations at NAQFGG, 3 presentations at TAPPI and 52 presentations: 10 invited and 42 voluntary, at the SFTIC conference. The conference had 200 registered attendees. Forty two were international participants from eighteen different countries. The employment makeup was approximately one third from private industry, one third from state or federal government and one third from universities.

Three awards were presented at the SFTIC conference. The Tony Squillace Award, given for the best oral presentation based on content, style and use of visual aids, was awarded to Scott Merkle for his presentation *Development of Transgenic Yellow Poplar for Remediation of Mercury Pollution.* Congratulations to Scott Merkle for an outstanding presentation. The Bruce Zobel Award, given in recognition of the best oral presentation by a student, was awarded to Xin-Yu Li for his presentation *Somatic Embyogenesis in Loblolly Pine.* Congratulations to Xin-Yu Li. In addition to the paper presentations, 22 posters were exhibited during the conference. The Belle Baruch Foundation Award, given for best poster, was awarded to H.V. Amerson and A.P. Jordan for their poster entitled: *Genetic Basis of Fusiform Rust Disease Resistance in Loblolly Pine.* Congratulations to H.V. Amerson and A.P. Jordan.

This year for the first time participants contributing voluntary papers to the SFTIC conference were given the option of submitting a full, ten page manuscript with citations and data or an extended abstract with citations (up to three pages). Of the 42 voluntary papers, 17 chose to submit an extended abstract. Extra copies of this proceedings can be ordered by writing or calling the National Technical Information Services, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, Tel #703-487-4650. Ask for publication # PB 97-186217.

The 24th Biennial Southern Forest Tree Improvement Conference committee would like to thank all participants, speakers, and moderators for their contribution to a successful conference.

24th SFTIC Conference Committee:

John Davis	*Bailian Li*	*Bob Schmidt*
Kim Gilmore	*Greg Powell*	*Steve Wann*
Dudley Huber	*Don Rockwood*	*Tim White*

24th Biennial Southern Forest Tree Improvement Conference

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PRODUCTION FORESTRY INTO THE 21ST CENTURY
A WORLD VIEW

R. C. Kellison[1]

Abstract. Until about 1960, industrial forestry and wood manufacturing was primarily in the northern hemisphere, notably in Scandinavia and North America where huge quantities of natural forests existed. Since then there has been a movement to the lower latitudes where exotic pine plantations supplied the resource. In more recent years, the emphasis has turned to establishment and use of plantation hardwoods. Among the reasons for this shift is the rapid growth rate of hardwoods, the timber of which can be harvested at about 6 years, and the great emphasis on use of printing and writing grades of paper, especially computer and copy paper. The US has been caught up in this trend, to the extent that hardwoods are being advocated on some lands that are better suited for pine plantations. Except for localized conditions, the message is to suppress this urge. For a given level of management, the softwoods will produce more wood per unit area and time, and their long fibers (tracheids) connotes strength properties to pulp, paper, paperboard and solid wood products that are lacking with hardwoods. Recycling will provide a brief respite to the timber supply dilemma, both in the US and worldwide, but an increased supply of primary (virgin) fibers will still be needed to satisfy the growing population and the increased per-capita consumption of that population.

INTRODUCTION

Until about 1960, the northern hemisphere had almost completely dominated the wood-based pulp and paper industry. That activity was particularly strong, as it continues to be, in Scandinavia and North America. Since that time, however, new activity has shifted to the lower latitudes, first to Australia, New Zealand and southern Africa, and then to Latin America, especially to Brazil. That activity continued its shift in Latin America, to Argentina, Venezuela, Columbia and, more recently, to Chile. But in these commodity traded wood products, no one claims dominance for long. The shift is now to the Far East, especially Indonesia and Southern China, but other countries in the region are being swept into the momentum.

The concentration of wood-based pulp and paper mills was once determined by the available resource. Huge quantities of natural timber existed in Scandinavia and North America. As the timber was harvested, reasonably good attempts were made to replace the forests, largely by plantations. Thus, the timber industry has continued to thrive in those regions. More recently, however, the emphasis has been to locate the manufacturing plants in areas where plantation forests exist; thus, the movement to Australia, New Zealand, southern Africa, Latin America and, now, Southeast Asia.

[1] Champion International Corporation, 1316 Dixie Trail, Raleigh, NC 27607

My purpose is to show where the world's greatest forest and wood-product manufacturing activity is located, and how that scenario will change in the early part of the 21st century.

THE US SITUATION

The per capita consumption of wood in the United States, for all purposes, is about 80 ft^3. That value has stayed relatively constant over the last decade whereas that of the paper and paperboard subset has declined during that time, from 699 lb. in 1988 to 680 lb. in 1995. During that period, however, the population increased from 245 to 263 million people[2]. Thus, more wood is being consumed each year from a forest land base that continues to decline in area and in quantity of available timber harvested. For example, the commercial forest land base in the South is about 180 million acres today, compared to about 200 million acres 25 years ago. In the Pacific Northwest, erosion of the land base has been less of a concern than is the amount of timber being harvested on public lands which represents nearly one-half of the productive forest land base in that region. Because of environmental concerns, public perception and governmental decree only about one-fourth of the timber is being harvested on the region's National Forests as was harvested in 1988. So, what is the future?

The US has, for decades, been a net importer of wood and wood products, ranging from 10% to 15% of total needs. The majority of those imports have come from Canada from where as much as 60% of our newsprint consumption has been received. In addition, about 33% of the structural lumber, from a total of 48 billion bd ft, comes from north of the border. Indications are, however, that the wood flow from Canada will steadily decline due to high manufacturing costs, distance to markets of both raw materials and products, US-imposed restrictions on imports and self-imposed forestry practices.

The short-term solution has been to shift more production to the US South where private ownership (only about 10% is publicly owned), climatic conditions, topography, infrastructure, labor supply and tolerance of forest farming is superior to other regions of the US and Canada. About one-third of the lumber consumed in the US is produced in the South, compared to about 21% in 1988. In addition, about 65% of the pulp produced in the US is of southern origin, and facilities for manufacturing engineered wood products, consisting of laminated veneer lumber (LVL) and oriented strand board (OSB), are continually locating to the area.

The question is "can the South sustain the harvest?" Starting about 1930 and continuing to this day, timber growth has exceeded drain in every region of the US, especially in the South. About 10 years ago, however, some analysts concluded that the softwood supply would be inadequate if all mills of solid wood products and pulp were operating to capacity. At about 53% of the total wood standing inventory, the further conclusion was that the hardwood supply would be inadequate, not because of the absolute lack of the

[2] The population increase is about 1.5 million annually, with about 1.1 million being immigrants of which 800,000 are legal.

resource but because of non-availability. As opposed to the softwood resource where about 50% of the potential supply is in 28 million acres of plantations, the hardwood resource is almost all in natural stands which are increasingly found in habitats where harvesting is limited by wet conditions or by steep and rocky terrain.

Forest industry, which owns about 22% of the commercial forest land in the region, has heard the call about the potential wood shortage. Whereas today with only about 30% of the consumed wood coming from fee and controlled land, a segment of the industry, including Champion International Corporation, has concluded that it needs to double that amount from fee and leased land and from contractual arrangements with non-industrial private forest owners. To accomplish that goal, on a land base that is being consolidated to those parcels of potential highest productivity and nearest the mills, wood productivity will have to be greatly increased. For example, Champion has a goal of increasing productivity by three times in plantations established in 2015 compared to those established in 1994. Other industrial organizations have similar goals.

The increased productivity will be obtained while adhering to sound sustainable forestry practices. To be good stewards of the land, and responsible to society, Champion has categorized its land base into four segments: **preservation**, **restricted**, **general** and **intensive**. The **preservation** category represents lands that are 'set asides' as special places. The will be protected from timber harvesting and other man-caused disturbances. The **restricted** areas constitute places such as streamside zones where selective harvesting of the timber may be practiced, but without the entrance of logging or site preparation equipment. The **general** areas are those where natural regeneration of the forest will be practiced; they will have special application to hardwood forests and to situations where it is appropriate to establish conifers by this method. It is the last category, **intensive**, where we expect to make the greatest impact in increasing wood production. We will farm those lands for timber, using every silvicultural practice that is economically and sustainably viable.

EFFECT OF RECYCLED FIBERS ON TIMBER SUPPLY

Recycling in the US had been a minor component of the total fiber supply until about 10 years ago. About 25% of the paper and paperboard produced had historically been collected for reprocessing, but 5% to 7% of that was regularly exported. The environmental concern about landfills being inundated with paper, paperboard and wood refuse created a political reaction that resulted in decrees to recycle a greater proportion of the 90 million tons of paper and paperboard produced annually in the US. The decree resulted in new de-ink mills being built, and for many existing primary-fiber mills to either begin using recycled (secondary) fibers or to expand its use. As a result, collection of the pulp and paper refuse now exceeds 45% of that produced, and the goal is to achieve a 50% recovery level soon after the turn of the century. A recovery rate beyond 50% becomes problematic because of even-flow and economics.

The use of secondary fibers does not mean that there will be a reduced, or even a static, use of primary fibers. Among the reasons are that, with each use, the fibers are fractionated, making them shorter and stiffer. Thus, each use relegates them to a lower-grade paper or paperboard until they eventually find their way into the effluent. For the highest-grade papers, secondary fibers can only go through the process about twice before grade specifications are violated. Even for the lowest grade of paper or paperboard, a repeat of the process for 6 or 7 times is the limit.

However, the task has not been achieved without its limitations. Because of fluctuations in market price of the finished product, from both primary and secondary fibers, the waste-paper flow has been erratic. During times of poor finished-paper prices, warehouses are filled with wastepaper, awaiting processing. At other times, when the price for the finished product is high, the waste-paper supply becomes exhausted, causing prices to escalate so that primary fibers are cheaper to process. It will still be a number of years before the combination of primary and secondary fibers finds an economically workable medium.

ALTERNATIVES TO INTERNALLY PRODUCED WOOD

The trend in recent years to produce greater quantities of printing and writing grades of paper has caused an increased use of. the broadleaved species, commonly known to us as hardwoods. The fibers of those species are one-third to one-fourth the length of the tracheids (fibers) of conifers, and the cell wall thickness of the fibers are proportional to their length. These wood properties translate to excellent paper-sheet formation and opacity[3]. They are also very well suited for the manufacture of boxboard (food cartons and ovenables, for example), and for the top layer (white top) on cartonboard which is commonly used for set-up advertising displays and advertising on shipping cartons.

As a result of the quality characteristics of the fibers, most pulp mills in the southern US have increased their use of hardwoods in the furnish. The concerns about long-term availability of the resource from natural forests have caused some organizations to invest in hardwood plantations in the Tropics and Subtropics. Among those organizations are Stone Container Corporation and Simpson Paper Company with *Gmelina arborea* plantations in Costa Rica and Guatemala, respectively, and Champion International with plantations of *Eucalyptus grandis*, *E. urophylla* and their hybrid in Brazil. The wood produced will either be sold on the world market, shipped to resident mills in the southern US, or processed directly into pulp and/or paper at the offshore location.

[3] Opacity translates to light diffusion; printing on the opposite side of the page is largely restricted on paper with high opacity.

THE RUSH TO HARDWOOD PLANTATIONS

Within the last two decades, the worldwide trend in the construction of new pulp mills or modernization and expansion of existing facilities has been similar to that of the US South in using increased amounts of hardwoods in the furnish. In addition to the huge demand for printing and writing papers, especially in countries with developing economies, hardwoods are favored for their high mean annual increments which allow them to reach financial maturity in 6 to 8 years. The trend to plant additional areas of hardwoods to fulfill these objectives is nowhere better exemplified than in Brazil where some pine plantations are being replaced with hardwoods, primarily eucalypts, in areas north of about latitude 25^0S. The phenomenon is also common to southern Africa, and to a lesser extent in Chile, New Zealand and Australia.

The greatest forestry phenomenon in the world, however, is taking place in Southeast Asia, inclusive of India. With economies that are expanding at 5% to 10% a year, a population approaching 3.4 billion people, and a per capita consumption of paper and paperboard of about 25 lb which is increasing about 2 lb per person per year, the demand for wood resources and manufacturing facilities is tremendous. In Indonesia alone, licenses have been issued for the construction of 14 world-class pulp and paper mills. Due to the lack of finances, it appears that only 5 of those facilities will be operational by the year 2000. These 5 mills are in addition to 4 wood-based mills, some of which are approaching one million metric tons per year of bleached hardwood pulp at a single location, with plans to double production within three years.

The wood resource for the operational pulp mills in Southeast Asia is primarily mixed tropical hardwoods, but plantations of *Acacia mangium*, *A. crassicarpa*, *Gmelina arborea*, and *Eucalyptus* spp. are starting to feed into the system. Plantation tree growth rates of 25 m^3/ha/yr (5 cd/ac/yr) are being obtained at harvest ages of about 6 years. With genetic improvement, average growth rates of 35 m^3/ha/yr (7 cd/ac/yr) are anticipated.

The alternative to growing plantation hardwoods offshore for potential use in the southern US is to grow the resource within country, using intensive culture. The example has been set in the Pacific Northwest where plantations of hybrid cottonwood, primarily *Populus deltoides x P. trichocarpa* and in northern California where *Eucalyptus camaldulensis* are being grown. The difference between these highly successful plantations, and the marginal industrial ones of about 100,000 acres established in the southern US since about 1970 is that the trees are vegetatively propagated, grown in a weed-free environment, and receive measured amounts of nutrients and water through a drip irrigation system. Growth rates of about 7 cd/ac/yr are obtained, with harvest ages of 6 to 7 years.

Based on the experience in the PNW, trial plantings have been installed in various parts of the southern US, primarily evaluating cottonwood (*P. deltoides*), sycamore (*Platanus occidentalis*) and sweetgum (*Liquidambar styraciflua*). To accommodate the fertilization/irrigation (fertigation) regime, soils with a high sand content are desired. Thus, if the scheme proves successful and if environmental concerns, such as availability of

irrigation water, can be resolved the marginally productive Sand Hills could be supporting our next hardwood crop.

Before investing heavily in hardwood plantations in the southern US, however, we should take stock of reality. The majority of the available forest lands are suited for growing southern pines, primarily loblolly (*Pinus taeda*) and to a lesser extent slash (*P. elliottii*) pines, but only the very best lands (and those conducive to fertigation regimes) are suited for growing acceptable crops of plantation hardwoods. Research results have shown that 4 cd/ac/yr can be achieved by growing loblolly pine on those best sites by controlling competition and pests, and by applying nutrients at appropriate times. Even on the poorer sites, 2 cd/ac/yr can be achieved with a reasonable silvicultural regime, at a rotation age of 16 years. Now, compare that to hardwoods. On the best sites, we might be able to achieve the same production as for pines, but because of the sensitivity of hardwoods to site, the poorer sites will give progressively less yields than pines.

Plantation yields of hardwoods in the Tropics and Subtropics, with eucalypts, can exceed 10 cd/ac/yr with rotations of about 6 years; whereas, the yields of pines, in southern Brazil for example, is about 6 cd/ac/yr at a rotation of 16 years. A little arithmetic will show that growth and yield of pine plantations in the southern US is reasonably comparable to the growth and yield of pine plantations in Brazil, reaching 40% and 80% of the goal from the poorest to the best sites. For hardwoods, however, even the best sites in the US South will achieve only about 40% of the productivity of their Brazilian counterparts. The point is that the forest lands in the US South, compared to large parts of Latin America, are more suited to growing plantation pines than they are to growing plantation hardwoods. The recommendation, therefore, is to avoid heavy investment in hardwood programs in the southern US, except on alluvial soils where hardwoods prosper at the expense of pines, and where productive hardwood plantations can be established on upland sites to fulfill a local need.

Extensive plantations of fast-growing conifers (almost universally pines) are restricted to Australia, New Zealand, Chile, southern Brazil, southern Argentina and the southern US. The potential exists for southern China to be included in this elite group, but my personal observation is that plantations established there before about 1990 received so little silvicultural care that they are only marginally productive.

On a world scale, long-fibered pulp that emanates from conifers, sells for a premium to short-fibered pulp. The cadillac of long-fibered pulp is spruce/fir (*Picea* spp./*Abies* spp), but following it closely is pulp from Monterey (*P. radiata*), loblolly, slash, Caribbean (*P. caribaea*), patula (*P. patula*) and kesiya (*P. kesiya)* pines. During times of high pulp prices, the spread between northern bleached softwood kraft (NBSK) and southern bleached hardwood kraft (SBHK) can be as much as $100/ton; during down cycles, the spread is less, but it is still commonly greater than $40/ton. As a comparison, northern bleached hardwood kraft (NBHK) sells at a comparable price to that of eucalypts pulp during both good times and bad, both of which have less market value than NBSK and southern bleached softwood kraft (SBSK).

The conclusion from this is that softwood pulps have historically sold at a premium to hardwoods pulps, and there is no reason to expect the situation to change. After all, softwood pulps are highly desired for products requiring strength, such as for linerboard and sack papers, and a portion of the furnish (10% to 15%) of even the best hardwood pulp and paper mills requires some softwood pulp, largely to hold the mat together on the high-speed forming belts.

The softwoods (conifers) also have intrinsic values for structural lumber and other solid wood products that are largely lacking in the hardwoods. Work is in progress in some countries, especially in New Zealand, Chile and Australia, and to a lesser extent in South Africa and the southern US, to manage fast growing softwood plantations for sawlogs or plywood bolts. The regime involves two or more thinnings, together with pruning to heights of 18' to 25', with an harvest age of about 28 years.

Plantation hardwoods that are managed for solid wood products, such as teak (*Tectonia grandis*), paulownia (*Paulownia tomentosa*), black walnut (*Juglans nigra*) and red ceiba (*Bombocopsus quinata*) are of greatest value when slow grown (more than four annual rings per radial inch); thus the management regimes are matched accordingly. In recent times, however, efforts are being made to manufacture furniture-grade lumber from fast growing species, such as from *E. grandis*. The key to success of this endeavor is to process green logs, quarter saw them as opposed to flat sawing, and pay heed to the heat, moisture and timing regimes of kiln drying.

SUMMARY AND CONCLUSIONS

Mention has been made of all the high forest-producing areas of the world, save one, and that is Russia. That country has more than half of all the long-fibered species in the world and, yet, we have chosen to ignore it. But for good reason. The political climate and the economy, along with the lack of infrastructure for forestry and forest product manufacturing is so in disarray that it will be a minimum of 20 years before Russia will become a major player in world trade of these commodities. For example, the pulp output in 1996 compared to 1995 was off about 25%, not from lack of markets but from aging mills and a disarrayed administration.

The area of greatest forest and forest product activity in the next two decades will be Southeast Asia, inclusive of India. Entrepreneurs in that part of the world are establishing fast growing forest plantations and building world-class manufacturing plants to satisfy a population of about 3.4 billion where the economies are growing from 5% to 10% annually. The entrepreneurs are committed to fulfilling the home market; they do not consider themselves being in competition with major paper and paperboard producers in other parts of the world. The effect on world markets, however, is that vendors from outside the region will have a difficult time selling into the region unless partnering relationships are formed.

Outside of Southeast Asia, the area with greatest latent potential for fast growing plantations and product manufacturing is southern Brazil, Argentina, Uruguay and Paraguay. This opportunity complements Brazil's existing pulp and paper industry that is largely concentrated in the Tropical and Subtropical zone immediately south of the Amazon Basin. The multi-country area is blessed with an excellent climate for forest growth, and it is replete with a good labor supply and infrastructure. Depending upon local conditions, both softwoods and hardwoods can be grown with high expectations. A high percentage of the land is open, having supported a marginal cattle industry; thus allowing plantation establishment with minimal cost. Much of the land, almost all of which is privately held, has already been planted to trees, assuring an initial wood supply for manufacturing plants locating to the area.

About 7 million acres of radiata pine exist in Chile, New Zealand and Australia. Much of that timber is just reaching harvest age, and a high proportion of it, as logs, chips, lumber and pulp, is destined for foreign markets, specifically Japan and China. These countries will have a significant impact on world trade in wood and wood products during the next two decades.

The southern US has great potential for maintaining its place as an international player in the manufacture and sale of wood products in the coming years. The greatest potential lies in intensively managed pine plantations, where growth of 4 cd/ac/yr can be achieved on the best upland sites in concert with intensive silviculture. Even on marginal sites, growth rates of 2 cd/ac/yr can be achieved. Almost all of the domestic hardwood resource will continue to be obtained from natural stands, largely because of the high cost associated with the relatively low yields obtained from plantations. The alternative for obtaining hardwood fiber or pulp for processing in southern US mills is to establish plantations in the Tropics or Subtropics, from which the wood or pulp would be ferried to the US. Offshore plantations of hardwoods for use in the US already exist in Guatemala, Costa Rica and Brazil.

RADIATA PINE AS AN EXOTIC SPECIES

C. E. Balocchi [1]

Radiata pine (*Pinus radiata* D. Don) is one of the most extensively planted exotic conifer in the world, there are about 3.8 million hectares in plantations. Most of the plantations are in 5 countries; Chile, New Zealand, Australia, Spain and South Africa (Table 1).

Table 1. Radiata pine plantations worldwide

COUNTRY	PLANTATIONS (000 ha)	YEAR OF INTRODUCTION
CHILE	1.380	1887
NEW ZEALAND	1.338	1868
AUSTRALIA	642	1876
SPAIN	237	----
SOUTH AFRICA	66	1885
OTHER COUNTRIES	100	----
TOTAL	3.763	

The natural range of the species is restricted to 5 discrete populations in western North America. Three of these populations are in coastal California, USA; Año Nuevo, Monterey and Cambria with a total area not exceeding 5.000 to 6.000 ha (Burdon y Banister, 1970). The other two populations are located in two islands off Baja California in Mexico; Guadalupe and Cedros. The island populations are very small in size, specially Guadalupe, where only a few hundred trees are left (Burdon y Banister, 1970; Forde, 1964; Scott, 1962).

The role of radiata pine on different countries is described based on a survey sent to organizations and scientists worldwide. Radiata pine has been established in experimental

[1] Genetic Division Manager, Bioforest S.A., Casilla 70-C Concepción, Chile

plantations in many countries in the world, but it has been successful only in countries with Mediterranean climate. Summer rainfall promotes infection by *Dothistroma septosporum*. Climatic conditions of natural and exotic populations are presented on Table 2.

Table 2. Climatic information for radiata pine in natural and exotic populations

COUNTRY	ANNUAL RAINFALL (mm)	ANNUAL TEMP. (°C)	COLDEST MONTH (°C)	HOTTEST MONTH (°C)	ABSOLUTE MINIMUM (°C)
USA , California	420-700	13-15	10-11	16-18	- 7
AUSTRALIA	650-1300	11-14	0.4-6	24-30	- 10
CHILE	450-2500	11-15	7-12	16-21	- 10
N. ZEALAND	700-1500	8-13	3-6	13-17	- 10
S. AFRICA	900-1100	9-12	9-11	10-13	- 3

Radiata pine is the main plantation species in three countries; Australia, Chile and New Zealand. Information on total plantation compared to radiata pine plantations is presented on Table 3.

Table 3. Participation of radiata pine on the exotic plantations per country

COUNTRY	TOTAL EXOTIC PLANTATIONS (000 ha)	RADIATA PINE PLANTATIONS (000 ha)	PARTICIPATION (%)
AUSTRALIA	1,040	642	62
CHILE	1,818	1,380	76
N. ZEALAND	1,478	1,338	90
S. AFRICA	1,429	66	5
SPAIN	3,500	237	7

Annually, about 200,000 ha of radiata pine plantations are established. 50% of these plantations are new plantations and the other 50% are replacement of harvested plantations. Table 4 presents a projection of plantations per country, considering that the annual harvest programs and the rate of planting will be maintained for the next 10 years,

Table 4. Historical and projected plantation are of radiata pine per country

COUNTRY	ANNUAL PLANTING (ha)	AREA OF PLANTATIONS (000 ha) 1956	1986	1996	2006
CHILE	70,000	200	1,118	1,380	1,800
SPAIN	5,000	51	270	237	237
S.AFRICA	3,300	21	56	66	80
AUSTRALIA	17,000	122	621	642	600
N.ZEALAND	95,000	229	1,009	1,338	1,800
TOTAL	190,300	623	3,074	3,663	4,517

Because radiata pine is grown in a great variety of sites, expected growth is highly variable. Table 5 shows some predictions of average growth per country and some indications of maximum growth on the best sites (Lewis and Ferguson, 1993).

Table 5. Average and maximum annual growth of radiata pine per country

COUNTRY	AVERAGE ANNUAL GROWTH (m3/ha/yr)	MAXIMUM ANNUAL GROWTH (m3/ha/yr)
SPAIN	13	---
AUSTRALIA	21	43
N.ZEALAND	24	45
S.AFRICA	14	37
CHILE	25	46

Radiata pine is managed for the production of sawn timber and fiber. Rotation ages vary from 18 to 35 years. Information on rotation age per country and management objectives is presented on Table 6.

Table 6. Rotation age for sawn timber and fiber production in different countries

COUNTRY	ROTATION AGE (YR) SAWN TIMBER	FIBER
AUSTRALIA	35	30
CHILE	25	18
N.ZEALAND	25-30	--
S.AFRICA	35	--
SPAIN	35	30

The actual wood production of radiata pine is close to 50 million cubic meter per year. The main uses for this wood is sawn timber, pulp and paper, panels and others. Based on the projection for the next 10 year of establishment of radiata, the long term wood production should double in year 2036. Information on wood production, actual and projected is presented on Table 7.

Table 7. Annual wood production of radiata pine per country

COUNTRY	ACTUAL WOOD PRODUCTION (000 m3/yr)	PROJECTED (2036) WOOD PRODUCTION (000 m3/yr)
CHILE	18,548	45,000
SPAIN	2,000	3,000
S.AFRICA	486	1,000
AUSTRALIA	10,400	12,000
N.ZEALAND	17,000	43,000
TOTAL	48,434	104,000

The main uses of wood in different countries is presented on Table 8.

Table 8. Main uses of wood in different countries

COUNTRY	WOOD USES (%)			
	SWAN TIMBER	PULP & PAPER	PANELS	OTHERS
CHILE	50	40	4	6
SPAIN	55	45	0	0
S.AFRICA	82	0	1	17 *
AUSTRALIA	45	53	0	2
N.ZEALAND	60	25	10	5

(*): mainly poles

Finally, the main biotic damage present on radiata pine plantations in different countries is presented in Tables 9 to 13.

Table 9. Main animal damage in radiata pine plantations

COUNTRY	MONKEYS BABOONS	KANGAROOS POSSUMS	RATS MICE
AUSTRALIA		X	X
CHILE			X
N.ZEALAND			X
S.AFRICA	X		X

Table 10. Main insect damage in radiata pine plantations

COUNTRY	RHYACIONIA BUOLIANA	SIREX NOCTILIO	HYLASTER SPP.
AUSTRALIA		X	X
CHILE	X		
N.ZEALAND		X	X
S.AFRICA			X

Table 11. Main needle diseases in radiata pine plantations

COUNTRY	DOTHISTROMA SEPTOSPORUM	LOPHODERMIUM PINASTRI	NAEMACYCLUS SPP.
AUSTRALIA	X	X	X
CHILE	X		X
N.ZEALAND	X	X	X
S.AFRICA	X	X	

Table 12. Main stem diseases in radiata pine plantations

COUNTRY	DIPLODEA PINEA
AUSTRALIA	X
CHILE	X
N.ZEALAND	X
S.AFRICA	X

Table 13. Main root rot diseases in radiata pine plantations

COUNTRY	ARMILLARIA SPP.	PHYTOPHTHORA SPP.
AUSTRALIA	X	X
CHILE	X	
N.ZEALAND	X	X
S.AFRICA	X	X

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LOBLOLLY AND SLASH PINES AS EXOTICS

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Abstract. Loblolly pine, *Pinus taeda* L., and slash pine, *Pinus elliottii* Engelm. rose to some prominence as exotics in five countries outside their native U. S. A.; Africa, Argentina, Australia, Brazil, and China. The earliest trials in these countries were established about the turn of the century. Tree improvement programs were initiated where there was promise that planting would continue in the long term. However, these programs were often begun without thorough provenance trials and many misconceptions about the adaptability, particularly of loblolly pine, arose. This, and problems with pathogens, insects, and predators have resulted in reduced interest in plantation establishment and consequently, tree improvement of both loblolly and slash pine in Africa and Australia.

Slash pine has been more widely planted in Africa than any other southern pine because of its many desirable attributes. However, often another pine species will produce more volume. Loblolly pine was not widely planted until erroneous beliefs about growth and wood quality were dispelled by genetic testing. Both species have a reduced status at present because of recently recognised deficiencies, susceptibility to diseases, and predation by baboons.

Loblolly pine is planted widely in the higher elevations or latitudes of Argentina and adjacent areas of Brazil, while slash pine is favored on lower elevation, poorly-drained sites. Tree improvement programs have been initiated in both countries, but the adequacy of their genetic bases is questionable.

Both loblolly and slash pines were widely planted on the infertile coastal lowlands of southeast Queensland, Australia and required the addition of phosphate fertilizer for normal growth. Because the site requirements for loblolly pine were greater, it fell into disfavor and tree improvement was discontinued in the mid 1900s. Slash pine is still in use as a parent of hybrids (*Pinus elliottii* var. *elliottii* x *P. caribaea* var. *hondurensis*) which exhibit better growth than either parent while combining several of their complementary characteristics.

Both loblolly and slash pine are used for reforestation at low elevations in the subtropical region of the China from 21° N to 33° N. In the south slash pine is the better alternative. In the central subtropical zone loblolly and slash pine perform equally well while in the northern Subtropical zone loblolly pine performs best. A major goal of breeding programs for both species is to accumulate sufficient genetic resources to maintain a long-term breeding program.

Keywords: Loblolly pine, slash pine, exotics, Africa, Argentina, Australia, Brazil, China.

INTRODUCTION

Southern pines were introduced into a number of countries beginning around the turn of the century. Loblolly pine, *Pinus taeda* L., and slash pine, *Pinus elliottii* var. *elliottii* Engelm. were subsequently widely planted in some of these countries and continue to be a significant component of regeneration efforts in several countries today. Loblolly pine is native to the Coastal Plain and Piedmont of the eastern and southeastern USA from Delaware south to central Florida, west to central Texas, and north in the Mississippi valley to southeastern Oklahoma, Arkansas, and southern Tennessee (approx. 28 ° N to 39 ° 30' N and 75° to 97 ° W) . Slash pine (var. *elliottii*) occurs on the Coastal Plain of the southeastern USA from southern South Carolina to central Florida and west to southwestern Louisiana (28° N to 33 ° N and 79 ° 30' W to 91 ° W) (Critchfield and Little 1966). In their native USA loblolly pine is generally better adapted to moist, but well-drained soils while slash pine is adapted to more poorly-drained soils. Both species have been most successful as exotics when planted within the same longitudinal range (N or S longitude). However, they have been successfully planted to about 15° S by increasing the elevation at which they are planted and ensuring adequate moisture during the growing season. We discuss herein the history of introductions, status at present, genetic improvement efforts, and the outlook for the future, by country.

ARGENTINA

History of Introductions

Provenance trials for both loblolly pine and slash pine were initiated in 1968 on sites from Buenos Aires to 30 km south of Iguazú in northernmost Misiones. The early results from these trials encouraged the importation of commercial seeds of both species. The relative ease of handling slash pine in the nursery and establishing it in plantations as well as its better form and appearance led to its preference over loblolly pine during the period from 1970-1988. However, loblolly pine exhibited faster growth rates on well-drained sites and the Argentineans began to import commercial seeds from the best seed sources in North Florida and Southern Louisiana and to establish a second series of loblolly pine provenance trials in Misiones and northern Corrientes in 1982. The results from plantations and provenance trials led to the initiation of a tree improvement program and the realization that the genetic base of loblolly and slash pines would have to be increased to support breeding in the long term. Thus, in 1984, 120 open-pollinated slash pine families were introduced into Argentina in cooperation with the Cooperative Forest Genetics Research Program at the University of Florida, USA. In 1990, 44 half-sib families of loblolly pine from wind-pollinated seed orchards of the Northern Florida and Southern Louisiana provenance were introduced into Argentina in cooperation with the USDA-Forest Service. These were to provide guidance for future seed purchases. As part of the same cooperative effort more than 100 controlled-pollinations representing breeding populations from East Texas to Eastern North Carolina were introduced to broaden the genetic base of the breeding population. These did not include the favored provenances, but did represent the families which had performed best in progeny tests in the Southern USA. A second collaboration in the same

year with the USDA-Forest Service introduced more than 100 half-sib families from wind-pollinated seed orchards of slash pine from Mississippi and Florida provenances into Argentina (Schmidtling *et al.*, these proceedings).

Status of Loblolly and Slash Pines in Argentina

Loblolly pine and slash pines are adapted to approximately 1 to 1.25 million hectares in the Misiones, Corrientes and Entre Rios Provinces of Northeast Argentina. Slash pine is preferred on poorly drained sites which occur primarily in Corrientes and Entre Rios. Yields for slash pine on these relatively poor sites are about 15-20 m^3/ha/yr. On more fertile soils the rainfall patterns, and the rarity of low temperatures (primarily in Misiones) result in almost continuous growth of loblolly pine where trees from unknown provenances reach mean annual increments of 20-25 m^3/ha/yr. The best provenances of unimproved loblolly pine from Northern Florida and Southern Louisiana yield 28-32 m^3/ha/yr (Pers. Comm. Sra. Mirta Báez), while slash pine planted in Argentina exhibits no strong seed source differences. Slash pine was planted more frequently than loblolly from about 1970 to 1988. Currently, about 80% of new plantations are being planted with loblolly pine. About 282,000 ha total have been planted to loblolly (56%) and slash (44%) pines to date (Pers. Comm. Sra. Mirta Báez). Primary pests are defoliating ants (Atta spp. and Acromrymex spp.) and Sirex noctilio which recently arrived from Brazil (Pers. Comm. Sra. Mirta Báez). Planting stock arises from seed production areas and first-generation seed orchards in Argentina and from commercial seeds purchased primarily from the Southern USA.

Genetic Improvement

The first selections of loblolly pine were made from plantations in Argentina in 1986 under the direction of the Centro de Investigaciones y Experiencias Forestales (CIEF). Selections were made in plantations of unimproved northern Florida and southern Louisiana provenances in Argentina. The first grafted seed orchard of loblolly pine was established in 1988 and open-pollinated progeny tests of the selected parents were established. This grafted seed orchard was expanded in 1988-1989 with further selections from plantations of the northern Florida provenance in Argentina. At present, the preferred source of seeds for planting in Argentina is from improved seed orchards in the USA because straightness and branch habit are better. Seedlings from unknown provenances are no longer planted (Pers. Comm. Sra. Mirta Báez). The need to increase the genetic base of the breeding population for loblolly pine was perceived to be critical since the genetic sample from the best provenances was limited. However, private companies in the USA were reluctant to share gene resouces from these provenances or to allow collections from their lands (Rogers and Ledig 1996) and another strategy was adopted. In 1990, 100+ controlled crosses each for loblolly and slash pines from the breeding programs of the USDA-Forest Service were introduced for testing their potential in Argentina. Unfortunately, these breeding populations do not include the best provenances, but should provide some opportunity to enlarge the breeding population.

Outlook

Interest in both loblolly and slash pines for plantation establishment remains high. A breeding strategy has been adopted for loblolly pine which includes a breeding population of about 600 selections managed in 12 sublines of 50 selections per subline. Subline composition will favor northern Florida and southern Louisiana provenances (Báez this proceedings). Plans for slash pine tree improvement include grafting three seed orchards in 1997 and development of a breeding strategy.

AUSTRALIA

History of Introductions

Loblolly pine and slash pine (*P. elliotti* Engelm. var. *elliottii*) were introduced to Queensland in 1917 and 1925, respectively. Both became important species for planting in the early 1930s (Rogers 1957). Seeds for plantation establishment were deliberately imported from north of the Ocala National Forest in northeastern Florida until seed needs were met (after 1936) by collecting from seed trees in Australian plantations (Rogers 1957). Provenance trials for both loblolly and slash pine were initiated in 1955 using 13 provenances from the Southwide Pine Seed Source Study. Results were typical of such trials for both species (Nickles 1962). Differences among slash pine provenances were minimal (at least through 1962), but loblolly provenances exhibited variation. The best provenances were from the coastal lowland sources from the southern USA with the best being from North Florida (Nikles 1975).

Status of Loblolly and Slash Pines in Australia

Both loblolly and slash pines were planted on sites not suitable for the native Hoop Pine (*Araucaria cunninghamii*) to augment the softwood supply (Rogers 1957). The sites on which they were planted were the infertile coastal lowlands of southeast Queensland and in frost pockets because seedlings of Hoop Pine were not frost resistant. Both exotic species required the addition of phosphate fertilizer for normal growth, but the requirements for loblolly pine were greater. The total area planted with loblolly pine approached 4,000 acres (Rogers 1957), but by 1952 only about twenty acres per year were being planted with that species (Nikles 1962) because it exhibited worse symptoms of phosphate deficiency than slash pine (Haley 1957). The number of acres planted with slash pine had risen to about 21,000 in 1956 (Rogers 1957) and it was planted until the 1980s when it was replaced by *P. caribaea* Morelet var. *hondurensis* Barrett and Golfari which grew much faster on all but the most poorly drained sites (Toon *et al. 1996*).

Genetic Improvement

The genetic improvement of loblolly pine began in 1941 with the establishment of the first clonal seed orchard and open-pollinated progeny trials of parents selected in plantations in Australia (Nikles 1962). By 1953 it was virtually dropped from the planting program and the

tree improvement program because of its poor performance on phosphate-deficient sites (Haley 1957).

The first slash pine seed orchard was grafted in 1953 using selections from stands planted in Australia in 1941. Genetic trials of both open- and control-pollinated progenies were established (McWilliam and Florence 1955) and a second slash pine seed orchard was established in 1957 when results were available from progeny tests (Nikles 1962).

Hybrid matings were attempted between slash pine and loblolly, palustris, radiata, and patula, but only slash x loblolly was successful (in 1957). In March, 1957 apparently fertile seeds were collected from a slash x caribaea cross (Haley 1957). *Pinus elliottii* var. *elliottii* x *P. caribaea* var. *hondurensis* hybrids were tested in 1958-1962 and exhibited better growth than either parent while combining several complementary characteristics of the parents (Nikles 1995).

Pinus caribaea var hondurensis replaced slash pine during the 1980's on all but the most poorly-drained sites and slash pine tree improvement languished until it was revitalized to support the hybrid program (Toon *et al.*)

Outlook

Three populations of slash pine are being maintained: 153 first- and second-generation parents (and their progeny) which are represented in genetic tests; 300 new first-generation parents selected between 1993 and 1995 in 30-year-old-plantations; and wind-pollinated seeds from 216 individuals selected in 25-30-year-old plantations for gene conservation (Toon *et al. 1996 a*nd Dieters and Nikles these proceedings).

BRAZIL

History of Introductions

Loblolly and slash pine were introduced into Brazil in the late 1940s on an experimental basis. In the early 1950s both species were planted on sites formerly planted to Araucaria. The first provenance trials were initiated in 1972-73. These included slash pines (*P. elliottii* var. *elliottii* Engelm.) from Coastal Louisiana, Alabama, Georgia and South Carolina and Florida. Loblolly pine provenances from the entire range of the species west of the Mississippi River were tested. In 1978, 55 seedlots representing good general combiners of loblolly pine from the Southeastern United States were introduced along with half-sib seedlots of both loblolly pine and slash pine open-pollinated in seed orchards from land races of both species in Zimbabwe. In 1982, 66 open-pollinated slash pine seedlots were introduced into Brazil in cooperation with the Cooperative Forest Genetics Research Program at the University of Florida, USA.

Status of Loblolly and Slash Pines in Brazil

Fiscal incentives from 1967 through 1986 encouraged the establishment of most of the pine plantations in place. Current estimates are almost 1,000,000 ha of loblolly pine and almost 200,000 ha of slash pine established. The demise of the fiscal incentives effectively ended the planting of both loblolly and slash pines in Brazil except by the large companies and annual planting rates are about 10,000 ha for loblolly pine and 2,000 ha of slash pine (Pers. Comm. Dr. Jarbas Shimizu).

Loblolly pine is planted primarily on well-drained sites along the coastal plains in the Southern States of Paraná, Santa Catarina and Rio Grande do Sul. South Carolina coastal provenances are among the best in Southern Brazil while more Northern provenances, for example the Piedmont of North Carolina grow equally well in cooler, highland climates (Pers. Comm. Dr. Jarbas Shimizu). The best provenances of loblolly pines are from North Florida and South Louisiana. Slash pine is better adapted to wet sites in Paraná, Santa Catarina and exhibits no significant differences among provenances (Pers. Comm. Dr. Don Rockwood).

Plantations from genetically unimproved seedlings produce 18-20 m^3/ha/yr on 16-25 year rotations. Genetically improved (first generation) plantations produce about 7 ton/ha/yr more than unimproved material (42 versus 35 tons/ha/yr). Plantations from rogued first-generation clonal seed orchards produce about 50 tons/ha/yr (Pers. Comm. Sra. Mirta Báez).

Genetic Improvement

Genetic improvement programs are conducted cooperatively among private companies through the Instituto de Pesquisas e Estudos Florestais (IPEF) at the University of São Paulo, Piracicaba, SP. Supporting research is conducted by Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA). Few companies have established seed orchards and most of these are first-generation unrogued orchards (Pers. Comm. Dr. Jarbas Shimizu). However, the oldest among these established about 1978-80 have been rogued and provide seeds for plantation establishment. Beginning in 1990 selection began to establish a second-generation base population. The breeding strategy is to maintain populations derived from the better provenances separately from those derived from slower-growing areas.

Outlook

Since fiscal incentives for reafforestation ceased (after 1986), plantations other than those made by large companies have virtually ceased. Ten years have passed without significant increases in plantation area in Brazil and a timber shortage is forecast for Brazil by 2005 (Pers. Comm. Dr. Jarbas Shimizu). Planting genetically improved seedlings is one way to rapidly increase forest yields and will likely play an important role in averting or ameliorating the expected timber shortage.

PEOPLE'S REPUBLIC OF CHINA

History of Introductions

Loblolly and slash pines were introduced into the People's Republic of China in several stages from the 1920's to 1989. Early introductions were small amounts for experimentation. In 1974, large quantities of seeds, usually of unknown provenance, began to be introduced for plantation establishment. The use of seeds of unknown seed source for plantation establishment arose because of a lack of information from provenance tests and because the availability of seeds in the People's of China was limited. In 1981, ten unimproved seed sources, identified only by state and county, were established in provenance trials in seven locations in China. A larger provenance trial was planted at eighteen locations in 1983. This second provenance trial included 31 lots of bulked seeds from first-generation seed orchards in the southeastern United States and 22 cold-hardy seedlots. In 1988 and 1989, a limited range collection was introduced. Fifty lots of wind-pollinated seeds from one wind-pollinated, Australian seed orchard and fifty wind-pollinated seedlots from two Zimbabwean first-generation seed orchards were introduced and established in 1989 in half-sib genetic tests. Seeds from 87 slash pine controlled crosses representing 41 unrelated parents and 83 loblolly pine controlled crosses representing 112 unrelated parents were introduced into the breeding populations in 1995 from selected parents that were progeny tested in the southern USA by the USDA-Forest Service. An additional 111 seedlots of loblolly pine and 126 of slash pine not previously tested in the USA were introduced into the breeding populations in 1996.

Status of Loblolly and Slash Pines in the China

Loblolly pine is planted in China in the southern, central and northern subtropical zones of the China from 21° N to 33° N. In the southern subtropical zone (21° to 24° N) slash pine is widely planted although loblolly pine may be a better alternative (Pan 1995), but in the large, central and northern subtropical zones (25° to 33° N) loblolly pine grows better than both slash pine and the indigenous Masson pine (*Pinus massoniana L.*) at elevations greater than 500 meters (Pan 1989). By 1992, about 1.5 million hectares of loblolly and slash pine plantations had been established in the proportions 20% and 80%, respectively (Pan 1995). However, loblolly pine performs better on many sites which have been planted with slash pines (Pan 1995). Plans are to establish 200,000 hectares of plantations annually through the 1990s. Early (6 to 8 years) results from provenance trials indicate that seed source is important for loblolly pine, but not for slash pine. The best sources of loblolly pine for the Southern and Central Subtropical zones of the People's of China are northern Florida and southern Georgia, Alabama, Mississippi, and Louisiana south of 32° N and coastal Georgia and South Carolina to about 34° N (Pan 1995 unpublished report). The Piedmont or upper coastal sources from the same regions are best for planting in the northern Subtropical zone of the China where freeze damage presents problems.

Genetic Improvement

The Hongling slash pine seed orchard, established in 1965, was the first seed orchard in China. Selections were made from mostly from naturalized plantations established from the 1947 and earlier introductions. Loblolly pine tree improvement began in 1983 with the initiation of the Qiaotou seed orchard in Yingde county. Plans for plantation establishment cannot be met from projected seed orchard yields and more hectares of both loblolly and slash pine are under establishment. New seed orchards promise substantially improved genetic gains since they will be established with the benefit of data from provenance trials and progeny tests planted in the 1980's. Seed supplies for slash pine are adequate at present because slash pine was the preferred species until field trials demonstrated the superiority of loblolly pine in the Central and Northern Subtropical Zones, particularly at elevations greater than 400 m (Pan 1989). For the same reason, loblolly pine seeds are now in greater demand and new seed orchards are being established to meet the expected demands. Seeds are still being purchased from abroad primarily because the older, most productive seed orchards were derived from land race plantations from early introductions and do not perform as well as unimproved seeds from the best provenances (Pan 1989). More hectares of slash pine seed orchards are being established to provide better genetic material for replanting plantations that will begin to be harvested within the next 10 years. Breeding strategies for both species have been adopted to ensure the continued genetic improvement of seedlings for plantation establishment in China.

Outlook

The outlook for both loblolly and slash pine in China is promising. Fusiform rust, *Cronartium quercuum* (Berk.) Miyabe x Shirai f. sp. *Fusiforme,* is apparently not a problem. However, *Diplodia* spp. has caused significant damage in plantations in the Northern Subtropical zone of China and *Oracella acuta* Lobdell has caused significant defoliation in southern plantations. *Dioryctria splendidella* H.S. is also an insect pest on loblolly and slash pines in China. The future success of loblolly and slash pine plantations in the China will depend in part on controlling both of these pests.

SOUTHERN AND EAST AFRICA

History of Introductions

The four principal southern pine species, loblolly pine, slash pine, longleaf pine, *P. palustris* Mill., and shortleaf pine, *P. echinata* Mill., were first introduced into the southern and east African region through South Africa around the turn of the century. The earliest record is of longleaf pine being planted in 1884, followed by loblolly pine later in that decade, shortleaf pine in 1899 and slash pine in 1918 (Poynton, 1979). Seed of these four species was later imported for establishment of trial plots in Southern Rhodesia (now Zimbabwe), loblolly pine in 1920, slash pine and shortleaf pine in 1929 and longleaf pine in 1930. Introductions were also made into Zambia, Malawi, Tanganyika (now Tanzania), Kenya and Uganda and also

Mozambique and Angola, mostly with seed from South Africa. The other southern pines, *viz.*, *P.glabra*, *P. pungens*, *P. rigida*, *P. serotina* and *P. virginiana*, were not introduced into the region until the mid 1960s when there was a concerted effort to carry out comparative tests of representative provenances of all the southern pines (Prevôst *et al.*, 1973a & 1973b; Mullin *et al.*, 1978). However, by this time, there were well-established breeding programs for slash pine and loblolly pine in the region and major gains in productivity and stem and branch form had been made through selection of plus trees in the early plantations.

Status of the Southern Pines in Southern and East Africa

Slash pine has been more widely planted in the region than any other southern pine. Provided it is not off-site, particularly at higher altitudes, slash pine has a long list of desirable attributes compared with other pines used operationally in the region. It is adaptable to a wide range of sites and tolerant of low soil fertility and poor drainage; it is relatively resistant to frost, fire, browsing by animals, *Hylastes* spp., *Pineus pini, Cinara cronartii, Eulachnus rileyi* and *Sphaeropsis sapinea*; it is tolerant of grass competition and high stocking and responds to delayed thinning; it is easy to establish and has a light crown habit that minimizes the problems of replanting in the slash; stem and branch form are good in bred populations and wood density high when planted at lower altitudes and these attributes result in the production of high quality lumber and high yield of pulp with good strength characteristics on short rotations (Morris *et. al.*); there is a high yield of oleoresin and tall oil production at the pulp mill. Its silvicultural disadvantages are that it is susceptible to *Armillaria mellea* and to bark-stripping by baboons and it is slow to capture the site. The resinous timber makes the species unsuitable for the production of groundwood pulp and resin-filled heart shakes can develop and seriously degrade the timber of rotation age trees (Darrow, 1982; Christie and Tallon, 1991). However, its most serious deficiency is that in almost any situation in which it will grow, there is always another pine species that will out-produce it and, although that species may have many other comparative disadvantages, it is usually productivity that wins the day when it comes to species choice.

Slash pine is silviculturally well adapted to climatic and edaphic conditions at the lower altitudes in the higher rainfall parts of the eastern escarpments in southern Africa. At the higher, coolest altitudes, however, where mean annual temperature is below 16.0°C, it is severely out-yielded by *P. patula*. However, its comparatively high tolerance of drought, frost, poor drainage and infertile soils gives it an edge over other species where these conditions occur singly or in combination.

Loblolly pine has been a neglected species in southern Africa from the time of its earliest introduction. The reasons for initial neglect were manifold and included an erroneous belief that it was tolerant of a wide range of sites; an ignorance of the importance of provenance in achieving the best performance of the species; excessively poor stem form and coarse branching of unimproved stock, especially when planted at high altitude; and an ill-founded reputation for the production of brittle timber that was unsuitable for sawn timber or pulp. Genetic improvement programs that included provenance testing and selection and breeding plus a better understanding of site requirements dispelled most of these reservations by the 1970s. It

was then seen as having many desirable attributes including very high volume production on the right site, good stem and branch form from bred material, resistance to *Sphaeropsis sapinea* and to *Hylastes* spp. and good litter breakdown on the higher, cooler sites. Disadvantages were seen as its susceptibility to drought death, *Pineus pini* and *Cinara cronartii*. Recently two additional concerns caused the virtual cessation of planting of the species. These were firstly, the development of a large number of "bottle-shaped" trees in South African plantations with associated abnormal wood formation that seriously affected utilization of the logs for sawnwood or pulp and secondly, the sudden discovery by baboons that the cambium on the main stem just below the live crown was palatable and an alternative source of food in the plantation environment where their natural sources of food had been exterminated.

Loblolly pine is well adapted to the better sites over a wide range of altitudes in the Eastern Districts where mean annual rainfall is over 1200 mm, preferably with an average of at least 25 mm in the driest months. Unlike *P. tecunumanii*, *P. oocarpa* and *P. maximinoi*, this species will tolerate frost. Provided the soils are also good, the yields will increase with decreasing altitude in relation to *P. patula* on similar sites but on the best high altitude *P. patula* sites, loblolly pine is unlikely to out-yield that species in the short rotations (< 25 years) currently used.

An estimate of the areas planted (in hectares) to the two operational southern pines, slash pine and loblolly pine, in southern and east Africa today are given below.

	Slash Pine	Loblolly Pine
South Africa	148,000	46,000
Zimbabwe	8,000	4,000
Swaziland	13,000	3,000
Malawi	4,000	100
Total	173,000	53,100

There have also been small commercial plantings in Kenya, Zambia, Tanzania, Angola and Mozambique, but the areas are small and unlikely to affect materially the total areas given above for the region. Slash pine probably constitutes about 23% and loblolly pine about 7% of the total softwood plantation area of southern Africa today.

Genetic Improvement

Provenance trials were planted in South Africa (Poynton, 1979), Zimbabwe (Prevôst *et al.*, 1973a & 1973b; Mullin *et al.*, 1978) and Malawi (Burley, 1966) in the 1960s. These trials soon showed the great importance of provenance in loblolly pine with the southern-most provenances hugely more productive than the northern provenances and these were not likely to have been represented in the original plantation from which the founders of the breeding populations had been chosen (Mullin *et al.*, 1978). However, the stem and branch form of these southern provenances was so poor that there was no question of immediate inclusion in the

breeding programs; and the strategy has been to import selected material from breeding programs in Florida and plant the material as resource stands in which to make selections later. Provenance variation was not so marked in slash pine although with this species as well, the southern provenances were generally demonstrably more productive, What the provenance trials did show was the folly of importing seed for operational planting from provenances of unproven performance. Between the time that Zimbabwe was importing seed from South Africa and producing its own from clonal orchards, there was a period when large amounts of both species were being imported from general collections in natural stands of uncertain origin. In loblolly pine progeny tests at seven years, controlled crosses from the bred material were eight times as productive as the imported material (Mullin *et al.*, 1978); much of this difference was due to suppression in the line plots of the test, but the mean height of the bred was twice that of the imported material at two years - before competition had set in. The Zimbabwe story with slash pine is very similar. The differences between the bred and imported material were not as great as they were in loblolly pine, but were still of sufficient magnitude to have highly significant economic implications.

Breeding programs for exotic pines were started in South Africa (van der Sijde and Denison, 1967) and Zimbabwe (Armitage and Barnes, 1966) in the late 1950s with the selection of plus trees, the establishment of clonal seed orchards and planting of progeny tests. The plus trees were selected in rotation age plantations that were planted in the early 1930s or earlier and therefore, at the time, there was no certainty that the material was from the best natural provenance. However, the most serious deficiencies in both *Pinus elliottii* and *P. taeda* were poor stem and branch form and there were good prospects of being able to improve sawlog quality through selection for stem straightness and fine branching in a population that had had one generation of proven performance. This certainly proved to be the case and stem and branch form in the plantations raised from seed produced from the first generation clonal orchards was so much improved that neither trials nor even comparison stands were needed to demonstrate the improvement; and progeny tests also showed growth rate to be better (Mullin *et al.*, 1978). Well designed progeny tests indicated high levels of additive genetic variation for the traits controlling these important morphological traits for both slash pine (Pswarayi, 1993; Pswarayi and Barnes, 1994, Pswarayi *et al.*) and loblolly pine (Gwaze, 1996), However, having taken care of poor stem and branch form in one generation, the other more recently recognized deficiencies have assumed greater importance *viz.* slow growth, baboon damage and heart shake in slash pine and baboon damage, drought proneness, abnormal wood formation and susceptibility to *Cinara cronartii* in loblolly pine. In fact, collectively, these detriments have brought about not only the virtual demise of these two species as important elements in southern African plantations, but also a reduced status in the breeding programmes which have ended up with large areas of highly productive clonal seed orchards with no national demand for their seed. The future for both slash pine and loblolly pine as operational plantation species in southern Africa depends, therefore, upon being able to counter the disadvantages through silviculture or genetics to make them competitive with the tropical pines such as *P. patula, P.greggii, P. tecunumanii, P. kesiya* and *P. oocarpa.*

Outlook

The most serious problem with slash pine is its slow rate of growth. Selection for stem and branch form took precedence in the first generation of the breeding programs, and therefore it is likely that some gain could be made in growth rate if that were the prime selection trait in the second generation; but it is unlikely to give gains that will make the species competitive with its rivals. It is unlikely that any genetically controlled trait will be found that makes the tree less susceptible to baboon damage; this will be solved by managing the baboon population which will probably be necessary in the end because the animals will turn to other species if slash pine is not present. The resin-filled heart shakes are thought to be caused by encased nursery soil which distorts the tap root and aggravates shake (Darrow, 1982); and although the possibility of genetic control has been suggested (Christie and Tallon, 1991), shake is more likely to be controlled by good silviculture and correct siting. It seems, therefore, that growth rate is the crucial trait and it may be that the future for slash pine will be in hybrid combination with *P. caribaea* var. *hondurensis*. This hybrid is in full operational use in Queensland to the exclusion of all other tropical pines over a large area. The hybrid exhibits the site tolerance and some of the frost resistance of slash pine and the growth rate of *P. caribaea* var. *hondurensis*. Provided the wood quality is acceptable when grown under southern African conditions, it could be grown over almost the whole of the area that was originally planted to slash pine and this species would, in this form, contribute its many attributes to the low altitude pine plantations in the region. The hybrid between slash pine and loblolly pine has been made and tested in Zimbabwe (Barnes and Mullin, 1978), but there is no niche in which it can demonstrate hybrid vigour.

The most serious problem with loblolly pine has been the formation of bottle-shaped stems with associated abnormal wood that renders the trees useless for sawn timber or pulp. This problem has virtually stopped the planting of the species in South Africa. There is some argument as to whether this condition is caused by plant growth regulator changes in response to pruning and thinning (Herman, paper not found here) or whether it is a response to an attack by the black aphid, *Cinara cronartii*. The latter seems to be distinctly possible reason for the widespread occurrence of the abnormality because a count of annual rings shows that its first appearance in unpruned and unthinned stands in Swaziland coincided with the arrival of the aphid. If it is due to the aphid, the problem is likely to subside because a parasitic wasp, *Pauwesia* spp. as been successfully introduced for biological control and progeny tests in South Africa do show that resistance to aphid attack is under genetic control. Loblolly pine is more drought-prone than all the other pines used in southern Africa, but this should be controlled by proper site selection. This is the crucial issue because *P. patula*, the most important pine in southern Africa, grows best on the moist higher altitude sites. Loblolly pine would therefore have to out-produce *P. patula* on these sites if it is to be grown on a larger scale in the region. There is evidence that the material now coming from the loblolly pine breeding programmes will out-produce *P.patula* at the lower end of the latter's zone; and it will produce larger sawlogs if grown on a longer rotation. Its wide provenance variation might also be used to extend the altitude range over which it can be grown. It appears, therefore, that loblolly pine has the prospect of increasing very considerably in importance in southern Africa in the future.

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THE GENETIC IMPROVEMENT OF CARIBBEAN PINE (*PINUS CARIBAEA* MORELET) — BUILDING ON A FIRM FOUNDATION

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Abstract:– *Pinus caribaea* Morelet comprises three geographic varieties or subspecies – var. *bahamensis*, var. *caribaea* and var. *hondurensis*. Variety *hondurensis* incorporates substantial variation between provenances and individuals within provenance; however for the other two varieties, variation is primarily among individuals. As well, var. *caribaea* and var. *hondurensis* especially, have substantial complementarity of characteristics important in commercial plantation forestry. Furthermore, var. *hondurensis* is the fastest growing of the three varieties, and it has been hybridised successfully with the other two varieties, *P. elliottii*, *P. tecunumanii*, and *P. oocarpa*. Thus genetic improvement of *P. caribaea* can use and is using the wealth of genetic resources contained in some species of the slash—Caribbean—Central American pines complex.

P. caribaea is an important species for commercial plantation forestry throughout the tropics and subtropics, with over 1 million hectares established world-wide. The future of this species (and some of its hybrids) in commercial plantations seems assured. Nevertheless the future of the broad range of genetic resources of *P. caribaea* that has been assembled through a series of exploration and seed collection efforts, and established in many *ex situ* plantings, is not assured. The genetic resources of the species has been dispersed across a number of geographic regions and organisations. There is a need to develop a coordinated and collaborative approach to the future conservation and use of the genetic resources that have been collected and developed in *ex situ* plantings.

Keywords: *Pinus caribaea*, provenance variation, breeding strategy, gene conservation

Pinus caribaea Morelet is a very important plantation species. Over the last 20-30 years the plantation estate has rapidly expanded, such that there are now over 1 million ha of plantations world-wide. There is still the potential for further expansions in the *P. caribaea* plantation estate. The genetic conservation, testing and breeding of *P. caribaea* has been characterised by a high level of collaboration and cooperation over this same period. This has led to the establishment of a world-wide network of international provenance trials, the exchange of genetic material, and the development of strong relationships between diverse people and organisations. In this paper we will briefly describe the taxonomy and distribution of the species, the history of international collaboration in the collection and testing of genetic resources, the current status of breeding world-wide, some benefits of hybridisation, and the need for continued collaboration to adequately conserve and wisely use the genetic resources of *P. caribaea*. It is our contention that continued international collaboration is vital to the future genetic conservation and sustainable genetic improvement of this species and its hybrids.

TAXONOMY AND DISTRIBUTION

The name *Pinus caribaea* was first used by Morelet in publications dated 1851 and 1855 to refer to slash pine (now *P. elliottii* Engelm.) growing in the south-eastern USA; its distribution was thought to extend to Central America and Cuba (Anoruo and Berlyn 1993). This resulted in considerable confusion because the name referred to both slash pine and the

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Caribbean pines. Anoruo and Berlyn (1993) describe how Grisebach (in 1864), Seneclauze (in 1867) and Loock (in 1951) attempted to divide the pine species in Central America, Cuba and the Bahamas Islands into three geographic entities; however, Little and Dorman (1952) were the first to effectively separate slash pine from the Caribbean pines. *P. caribaea* was subsequently further separated into three varieties (*bahamensis, caribaea* and *hondurensis*) based on the independent work of Barrett and Golfari (1962) and Luckhoff (1964). The nomenclature of the species has remained unchanged since.

The natural distribution of the Caribbean pines lies between 27°25′N latitude in Grand Bahama and Great Abaco and 12°13′N near Bluefields on the east coast of Nicaragua, while the longitudinal range is from 71°40′W in the Caicos Islands to 89°25′W at Poptun in Peten province of Guatemala (Lamb 1973). However, continued exploration work by CAMCORE (Central America & Mexico Coniferous Resources Cooperative) continues to expand the known distribution of the species slightly. The three varieties of *P. caribaea* are found in three separate geographic regions:

- var. *bahamensis* on the Bahamas and Caicos Islands,
- var. *caribaea* on the western part of Cuba in the province of Pinar del Rio at Cajalban, and at the northern end of Isle of Pines, and
- var. *hondurensis* on the mainland of Central America (Mexico, Belize, Guatemala, El Salvador, Honduras and Nicaragua) and Guanaja and Roatán Islands off the northern coast of Honduras.

EXPLORATION, COLLECTION AND INITIAL DOMESTICATION

The first recorded exports of seed are from Belize to California and South Africa in 1927 (Luckhoff 1964). A stand of 2.4 ha was established in 1929 at Dukuduku (on the Zululand coast), South Africa at a latitude of 28°30′S (Lamb 1973). In 1930, a stand of *P. caribaea* was established in Queensland, Australia near Imbil at latitude 31°S which Nikles (1966) confirmed to be var. *caribaea*.

However, it was not until after World War 2 that more substantial and comprehensive introductions of *P. caribaea* were made. There were broadly two waves of post-war introductions. The first wave in the 1950s and 1960s, involved introductions of var. *hondurensis* and then var. *bahamensis* and *caribaea* primarily to Australia (Queensland) and South Africa. These introductions elicited great interest because of the superior growth of *P. caribaea* when compared to *P. elliottii* (Luckhoff 1964, Nikles 1962, Slee and Nikles 1968). In 1955 a small scale breeding program had commenced with var. *hondurensis* in Queensland (Slee and Nikles 1968), and in South Africa by the 1960s. Seed collections (and exports) were almost exclusively from upland sources of var. *hondurensis* from Belize prior to 1960 (Greaves 1978) and the establishment of the Tropical Silviculture Unit of the Commonwealth Forestry Institute (now Oxford Forestry Institute, OFI) in 1963 (Lamb 1973). The first replicated provenance trial that included the *bahamensis* and *caribaea* varieties was established in South Africa in 1959 (Lamb 1973). However, a provenance test of var. *hondurensis* planted in 1956 in Queensland contained one plot of var. *bahamensis* (Nikles 1962, Slee and Nikles 1968). These trials revealed the superior stem straightness of var. *bahamensis* and var. *caribaea* compared to var. *hondurensis*, and stimulated interest in the

further exploration and testing of these two varieties. Preliminary breeding programs did not commence with these two varieties until the late 1960s and early 1970s.

The recent history of the genetic improvement of *P. caribaea* has been characterised by collaboration. The second post-war wave of introductions was precipitated by the 1962 report of the Committee on Silviculture to the Eighth Commonwealth Forestry Conference — Greaves (1978) quotes the following resolutions passed by the Conference in response to this report:

> "(i) A special study be initiated into the races and provenances of *Pinus caribaea*.
> (ii) Countries interested should make arrangements for coordinated seed collection and provenance trials.
> (iii) The above projects be initiated and coordinated by the Commonwealth Forestry Institute."

Subsequently, the OFI with funds provided by most Commonwealth countries and the British Overseas Development Ministry (now Overseas Development Administration, ODA) collected seeds between 1963 and 1969 from natural stands of *P. caribaea* and *P. oocarpa* (Greaves 1978). Distribution of the *P. caribaea* seed commenced in 1971 from a total of 35 natural provenances (19 of var. *hondurensis*, 10 of var. *caribaea* and 6 of var. *bahamensis*) and one improved collection of var. *hondurensis* from a clone bank at Byfield, Queensland (Greaves 1980). Birks and Barnes (1990, page 1) state, "By the end of the 1970s many hundreds of trials [of *P. caribaea* and of *P. oocarpa* Schiede (incorporating what is now known as *P. tecunumanii* Eguiluz)] had been established with representation of 20 to 30 provenances of each species in over 50 tropical countries".

A second round of collections were initiated in the late 1970s and 1980s by OFI and CAMCORE respectively. The identity of individual open-pollinated families was retained and the results from the earlier OFI-sponsored provenance trials were used to help determine collection priorities (Crockford et al. 1990, Dvorak and Donahue 1992). The OFI collections were restricted to the most promising provenances (Crockford et al. 1989, 1990) with the objective of providing a selection base for the creation of breeding populations. However, CAMCORE sampled many provenances that had not previously been represented in international provenance trials including some remote/isolated sources, and OFI/ODA collected additional seed from Guanaja Island in the mid-1980s (Dvorak 1992). The DANIDA Tree Seed Centre was also involved in assembling and distributing *P. caribaea* seed from natural stands during this period (Nikles 1996). FAO facilitated the establishment of conservation stands of two provenances (Poptun and Alamicamba) of var. *hondurensis* in several tropical countries (Wood and Burley 1983). These new provenance introductions, and extra seed of some them, provided a much broader genetic basis for breeding (Nikles et al. 1983).

Between 1982 and 1993 CAMCORE sampled 23 provenances and 1178 mother trees of var. *hondurensis* in Central America and Mexico (Dvorak et al. 1993), and established 94 tests in six countries (Dvorak and Donahue 1992). CAMCORE has continued their exploration and collection work, with the first collection of *P. caribaea* from El Salvador occurring in 1996

and they now estimate that 99% of the genetic diversity of this species has been sampled (CAMCORE 1996, pages 1and 24).

As a result of the early work and these internationally-sponsored collections and establishment of trials, as well as other *ex situ* conservation facilities, there is an unprecedented wealth of genetic resources of *P. caribaea* var. *hondurensis* (and also *P. oocarpa* and *P. tecunumanii*) available for exploitation in breeding programs. Furthermore, in the 1970s and 1980s there were considerable exchanges of plus-tree seed and/or scions and pollen (e.g. Pottinger and Barnes 1989) among many of the organisations which developed active breeding programs with var. *hondurensis*. Thus, for example, the Queensland breeding populations of var. *hondurensis* now include a number of plus-trees selected in imported families (170) and imported clones (30).

Much of the exploration and seed collection work described above has concentrated on the more widely distributed var. *hondurensis*. Other than the early collections of Luckhoff (1964) and Nikles (1966) the genetic resources of var. *bahamensis* (particularly) and var. *caribaea* have, by comparison, been subject to only fairly limited exploration and collection. As noted above in the initial 1970's OFI collections 6 and 10 provenances of var. *bahamensis* and var. *caribaea* respectively, were sampled. However, the performance of only 1 and 7 provenances of var. *bahamensis* and var. *caribaea* respectively are reported in Birks and Barnes (1990). Subsequently, exploration and collection activities concentrated primarily on var. *hondurensis* because of its better growth in international provenance trials (Baylis and Barnes 1989, Birks and Barnes 1990).

In the late 1980's OFI, funded by the ODA, initiated a project to collect and distribute seed of var. *bahamensis* following recognition of the possible value of this variety (and var. *caribaea*) due to its greater insect and disease resistance compared to var. *hondurensis*. Tip moth has devastated some plantings of var. *hondurensis* in south-east Asia, and further spread could mean that var. *bahamensis* and var. *caribaea* (or hybrids with these varieties) may become the most important softwood species in the low altitude/latitude tropics (Baylis and Barnes 1990). In total, seed was collected from 10 individual trees in each of 14 provenances throughout 4 islands of the Bahamas. However, no seed was collected from the Caicos Is., the most southerly occurrence of var. *bahamensis*. Seed from most of these families was used to establish family-in-provenance studies on one site in southern China in 1991 (Zheng et al. 1994) and another site in south-east Queensland in 1990.

An extensive seed collection across the natural range of var. *caribaea* was undertaken by the Edinburgh Centre of Tropical Forests and the Institute of Ecology and Resource Management at the University of Edinburgh, in 1994 (Zheng 1966, page 2-31), as part of an ODA UK-China project. The overall aim was to form a base population for future breeding of this variety in China. Seed was collected from 195 trees in 12 natural provenances and one seed orchard population in Cuba (Zheng 1996, pages 2-31,32). This seed, along with land-races from Brazil and China, has been used to establish a base population of 220 open-pollinated families in southern China (Zheng 1996, page 6-145).

Gibson et al. (1983) and Birks and Barnes (1990) present comprehensive summaries of the results of the initial OFI international provenance trials with *P. caribaea*, and a review of the wood properties of var. *hondurensis* across 8 countries and 11 tests is given by Wright (1990). Crockford et al. (1990, chp. 7) provide a summary of the initial results from the var. *hondurensis* family-in-provenance studies. Numerous authors have reported the results of individual trials (mostly originating from OFI collections), for example: Slee and Nikles (1968), Brigden et al. (1983), Eisemann et al. (1983), Nikles et al. (1983), Haines (1984), Rider et al. (1984), Tozer and Haines (1984) and Wright et al. (1994) in Australia, Zheng et al. (1994) and Pan and Nikles (1996) in China, Bird (1984) in Costa Rica, Das and Stephen (1984) and Tavitayya (1984) in India, Otegbeye and Shado (1984) and Otegbeye (1988) in Nigeria, Kha et al. (1989) in Vietnam and Wright et al. (1986) in Zambia. A summary of the CAMCORE provenance trials established in Brazil, Colombia and Venezuela with var. *hondurensis* provenances from Honduras and Guatemala is given by Dvorak et al. (1993). A more comprehensive analysis of the CAMCORE tests has recently been completed by Dr. Gary Hodge; however, a copy of this report was not available at the time this paper was prepared.

In provenance trials where the three varieties have been compared the following general trends are evident:

- Variation between trees within provenance was at least as great as variation between different provenances.
- Little variation amongst provenances of varieties *bahamensis* and *caribaea* has been reported to date (Nikles 1996); however this may be due to less environmental variation across the range and/or to less intensive sampling of these varieties. However, by contrast substantial variation has been reported among var. *hondurensis* provenances.
- Across a range of sites and countries var. *hondurensis* consistently out-performs the other two varieties in terms of early growth. However, the growth of var. *bahamensis* may exceed that of var. *hondurensis* at slightly higher latitudes and/or altitudes (Luckhoff 1964, Gibson et al. 1983, Baylis and Barnes 1989).
- Varieties *bahamensis* and *caribaea* exhibit generally better stem straightness, greater resistance to wind-damage (as measured by stem lean), and a lower incidence of "fox-tails" than var. *hondurensis* (Birks and Barnes 1990). Note: there was only one var. *bahamensis* provenance (Andros) in the OFI-sponsored tests, and this provenance was similar to the var. *caribaea* provenances in stem straightness and lean (Birks and Barnes 1990). Brigden et al. (1983), Rider et al. (1984) and Pan and Nikles (1996) report similar findings for stem straightness.
- Variety *hondurensis* is markedly inferior to the *bahamensis* and *caribaea* in terms of resistance to insect attack. In Vietnam var. *caribaea* is reported to have superior resistance to insect attack (Kha et al. 1989). In China (Pan and Nikles 1996) var. *hondurensis* had a substantially lower survival (75% compared to near 100%) and higher susceptibility to tip moth (*Rhyaciona* and *Dioryctria* spp.) attack and brown needle disease (*Ceroseptoria pini-densiflorae*) than var. *bahamensis/caribaea* and slash pine. Baylis and Barnes (1989) and Zheng et al. (1994) also note varieties *bahamensis* and *caribaea* may prove to be more suitable for use in south-east Asia because of their resistance to tip moth attack.

Var. *hondurensis*: Birks and Barnes (1990) define three provenance regions of var. *hondurensis*: upland (UPL), coastal (COA) and island (i.e. Guanaja, GUA). In Queensland, these provenance regions can be clearly delineated in terms of wind-firmness (Nikles 1996): the COA provenances show considerably less wind-damage following cyclones than UPL provenances, while GUA material tends to be intermediate (Nikles et al. 1983). Coastal provenances on average also tend to be straighter than upland sources (Eisemann et al. 1983, Birks and Barnes 1990), but have a higher susceptibility to fox-tailing (Birks and Barnes 1990). In terms of growth rates, the Guanaja Island provenance performed well in the OFI trials (Birks and Barnes 1990, Crockford 1990) and provenances with good growth rates can be found amongst both UPL sources (e.g. Belize Mountain Pine Ridge and Poptun) and COA sources (e.g. Laguna el Pinar, Karawala, Alimicamba). The coastal provenance El Limon from Honduras (as distinct from the upland provenance Los Limones from Honduras, in the OFI-sponsored trials) has performed well on a number of sites in the CAMCORE tests (Dvorak et al. 1993); however, in Queensland the stem form of this provenance is inferior to a number of other high-growth provenances. All provenances of var. *hondurensis* tend to be susceptible to attack by tip moth in south-east Asia (Birks and Barnes 1990).

Considerable variation has been reported in wood density (DEN), and variation in wood density (VAR) between provenances of var. *hondurensis* (Wright et al. 1986, Birks and Barnes 1990, Wright 1990, Wright et al. 1994). The Guanaja, Poptun and Santa Clara provenances have consistently demonstrated above average density across a range of sites, and Guanaja had a very low variation in density (Wright 1990).

Var. *caribaea* and var. *bahamensis*: The OFI-sponsored tests reported by Birks and Barnes (1990) include only one provenance of var. *bahamensis*, and therefore provide no information on provenance variation in this variety. Generally, differences among var. *caribaea* provenances have not been found to be statistically significant for most economically important traits (Nikles 1966, Rider et al. 1984, Birks and Barnes 1990, Pan and Nikles 1996). The *bahamensis* variety tends to be more variable than var. *caribaea* as might be expected from its more disjunct distribution across 4 islands of the Bahamas and 2 of the Caicos Islands (Nikles 1996). For var. *bahamensis* growing in southern China, Zheng et al. (1994) found significant differences between region (i.e. islands), provenances within region, and families within provenances for all traits examined (height, diameter and crown width) at 2.5 years of age. Zheng et al. found a 11% difference in height and diameter between the fastest and the slowest growing regions, with the northern sources (Abaco Island) generally performing better than the southern sources. All three traits were significantly correlated with the latitude of provenance origin, and hence rainfall distribution (Zheng 1996, page 5-119). However, Zheng's study did not include the most southerly Caicos Island sources. Nevertheless, most (> 40%) of the variation was between individuals within provenances (Zheng et al. 1994), as has been noted above for *P. caribaea*.

BREEDING PROGRAMS

P. caribaea has been introduced to over 50 countries since the early 1970s; however, very few of these countries now maintain an active plantation program with the species. Tables 1

and 2 summarise the countries with major *ex situ* plantings of *P. caribaea* (all three varieties) and some aspects of the silviculture. Despite the potential to make large gains through breeding in this species (Nikles 1996), of those countries using *P. caribaea* as a commercial plantation species, even fewer are now actively involved in the genetic improvement of the species (Table 3). Further, it is clear that var. *hondurensis* is the most important variety in plantation forestry, followed by var. *caribaea*. There is clearly great potential for the commercial deployment of this species:

- Very large areas have been planted in South America (almost 1 million hectares, Table 1), and the total world-wide plantation area could eventually approach that of other major conifer species such as *P. radiata*.
- The *bahamensis* and *caribaea* varieties have much greater resistance to tip moth than var. *hondurensis*, therefore offering opportunities for the expansion of plantations with *P. caribaea* in east Asia. The plantation estate in south China is expected to increase 3-fold over the next 15 years (Zheng 1996, page 1-20), primarily using these two varieties.
- There is great interest in the use of *P. caribaea* hybrids, including inter-provenance hybrids, inter-variety hybrids, and inter-specific hybrids (Table 3). The use of some hybrids has the potential to increase the area over which *P. caribaea* and its derivatives may be deployed commercially.

Genetic gains from breeding: Dean et al. (1986) indicate that substantial genetic gains are possible from recurrent cycles of breeding with var. *hondurensis*. Subsequently, a number of other authors have also reported moderate levels of additive genetic variance associated with traits of economic importance (e.g. Woolaston et al. 1990, Zheng et al. 1994, Telles dos Santos et al. 1996, Vásquez and Dvorak 1996) and hence the opportunity to achieve economic gain through breeding.

Nikles (1996) highlighted the gains that have been demonstrated in the OFI-sponsored provenance tests. Both the first- and second-stage tests included an "improved" source of var. *hondurensis* that was derived from the early stages of the Queensland breeding program. Birks and Barnes (1990) reported: "The best performer [in productivity] across sites among the var. *hondurensis* provenances was the Byfield (Queensland) seed orchard which itself was derived from the Mountain Pine Ridge (Belize) origin" (Birks and Barnes 1990, page 32). This result is clearly demonstrated in Figure 1.

Breeding strategies: Broadly, there are currently three groups involved in the genetic improvement of *P. caribaea*: CAMCORE (active) members, countries associated with CIRAD of France and non-aligned organisations. CAMCORE is currently in the process of developing a cooperative, regionalised, multiple-population, breeding strategy which will allow individual members to participate in the improvement of *P. caribaea* var. *hondurensis* at varying levels of intensity (CAMCORE 1996). In the other two groups (within and between which some collaboration has taken place), the breeding strategies employed fall broadly into two classes: low and high intensity breeding. High intensity work is characterised by the maintenance of full-pedigree information, controlled crossing and intensive progeny testing. The low intensity breeding work is typified by the use of "breeding seedling orchards" (BSO), where the functions of breeding, testing, selection and seed production are combined in the one facility (Barnes 1984). The breeding program with var.

hondurensis and var. *caribaea* in Queensland would fall into the high intensity group, while work with var. *hondurensis* in Fiji, var. *bahamensis* in Queensland, and var. *caribaea* and *bahamensis* (Zheng 1996) in China would fall into the low intensity category. However, Zheng (1996) indicated that breeding with *P. caribaea* in China may become more intensive following one or two generations of improvement via the simple BSO strategy.

An additional group which could be added to the above three groups is the "Center of Genetic Conservation and Breeding of Tropical Pines" (CCGMT) in Brazil which is a cooperative association of university and industry groups (Telles dos Santos 1996). CCGMT maintains breeding programs of all three varieties of *P. caribaea*, grafted 150 ha of clonal seed orchards (which include all 1000 members of the breeding program), and established over 50 progeny tests in Brazil and Argentina (Telles dos Santos 1996). However, we are not aware of the current intensity of breeding activities within this program.

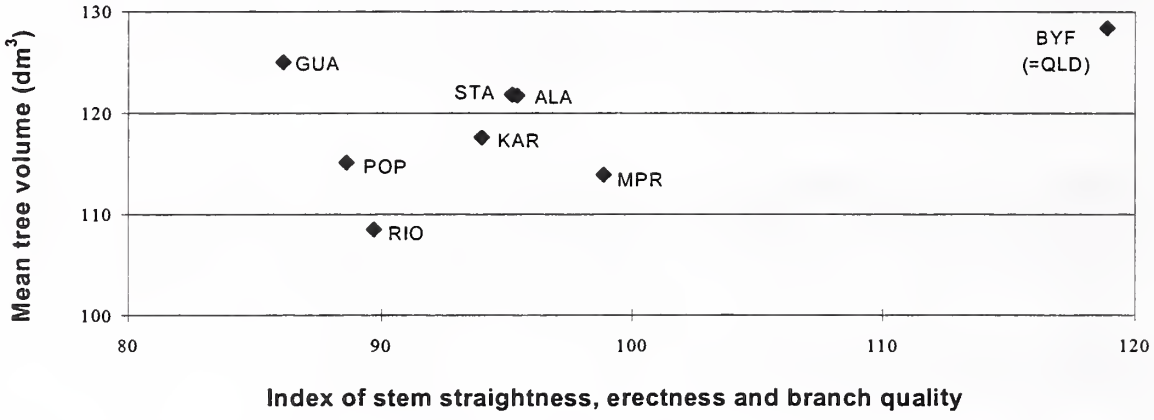

Figure 1: Relationship of provenance means across 9 tests world-wide for mean tree stem volume under bark (dm^3) and, across 11 trials, of an index of mean tree stem straightness and branching for provenances of *P. caribaea* var. *hondurensis* (ALA, KAR, RIO – coastal provenances; MPR, POP, STA – upland; GUA – island) and BYF, an early, Queensland (QLD) improved variety derived from MPR. Data based on 6 year assessments reported by Birks and Barnes (1990). Figure taken from Nikles (1996).

Constraints to breeding: There are a number of constraints to the breeding of *P. caribaea* throughout the world (Table 3). These constraints can be categorised as biological (seed/flowering problems, insect and disease resistance), abiotic (wind damage and fire), and infrastructure (staffing, funding, etc.).

Problems with seed production and flowering were investigated by Gallegos (1983). He concluded that optimal flowering and seed production occurred between 9–27° latitude (north or south), higher elevation sites within this latitude range had reduced flowering but that high elevation sites closer to the equator were more favourable for the production of viable seed. Slee (1977) also reports problems of needleless shoots and dieback often associated with abnormal flowering at low latitudes. Organisations such as SAFCOL (South Africa) have recently invested considerable resources in the development of seed production facilities at

lower latitudes (Mozambique) in an attempt to overcome seed production problems (Neville Wessells, *pers. comm.* 1996).

Nevertheless, from the experience with *P. caribaea* in Australia the situation is probably not as simple as that outlined by Gallegos (1983). For example: male and female flowering of var. *hondurensis* is asynchronous in the Northern Territory at latitude 12° S, seed production is very good at Cardwell (lat. 18°S), is poorer (though acceptable) at Byfield (lat. 23° S), but almost non-existent at latitude 26°S. Further, the varieties of *P. caribaea* and provenances within var. *hondurensis* exhibit differences in flowering and seed production: var. *caribaea* has a long delay following grafting until the on-set of flowering (over 5 years), and the coastal provenances of var. *hondurensis* also appear to be less precocious than the upland sources. These delays in the on-set of flowering have caused considerable problems in the implementation of the multiple population breeding strategy outlined by Kanowski and Nikles (1988), such that the generation interval for coastal sources will be considerably longer than the upland sources. Therefore, it is unlikely that the recently infused coastal material will "catch-up" to the more advance breeding population based on Belize Mountain Pine Ridge material.

Insect and disease problems seem to be principally restricted to south-east Asia. The main approach to solving this problem has to been to switch from the faster growing but more susceptible var. *hondurensis* to varieties *bahamensis* and *caribaea*. As Baylis and Barnes (1989) point out, if the tip moth of south-east Asia should spread further, then these two varieties could assume a much greater importance world-wide. The history of the often eventual international spread of insect pests means that all growers of *P. caribaea* should be aware of the potential risks, and perhaps invest some resources into the breeding and testing of *bahamensis* and *caribaea*. For example, Queensland has just established a 14 ha planting of var. *bahamensis* for gene conservation purposes, even though this variety *per se* is currently of no commercial interest in Queensland.

Wind-damage and fire have import consequences for breeding. Although, it has been possible to make good progress in the genetic improvement of wind-firmness in var. *hondurensis*, if progeny tests and clone banks are repeatedly damaged by fire and wind, it becomes increasingly difficult to maintain an effective breeding program (e.g. Fiji and New Caledonia, Table 3). Also, because all breeding facilities must be replicated to ensure that genetic resources are not lost in the event of severe wind-damage or fire, there are considerable additional costs imposed on the breeding program. Costs are associated with both the duplication of facilities and the opportunity cost associated with foregoing other (perhaps more productive) activities.

Infrastructure problems seem to be increasing as we move into the 20th century, as demonstrated in Table 3. Breeders are often faced with the need to accomplish more work with less resources. This continued squeeze on resources has important consequences for the improvement of *P. caribaea*. As described earlier, a wealth of genetic resources has been accumulated world-wide from the natural stands of *P. caribaea*. Many of the natural populations from which this material was derived have now been destroyed, greatly depleted since the original germplasm was exported, or likely to be threatened in the future. Further,

Table 1: Primary *ex situ* distribution of *P. caribaea* Mor. throughout the world. Information derived from survey results where indicated; otherwise, based on other information available to the authors.

Country	Date of Introduction (year)	Organis-ations (#)	Total Estate (ha)	Latitude Range	Longitude Range	Total Rainfall Range (mm)	Summer Rainfall Range	Days Below -2°C	Coldest Annual Temp.
Argentina[6]	1960-1970s	2?	?	≈ 25°-28° S	≈ 57° - 58° W	800 to 2400	–	?	-1.5°C?
Australia	1930s	2	≈58,000[‡]	12-26°S	132-154°E	1,000 to 2,000	700 to 1,500	nil	5°C
Brazil[1]	1950s	4?	300,000	1°N-27°S	49-52°W	1,000 to 1,500	800 to 1,200	nil	10°C (*c,h*) 6°C (*b*)
China[2]	1961 (*c*)[†] 1973 (*b,h*)	1	40,000[§]	18°55′-24°15′N	–	1,530 to 2,250	–	nil	
Fiji[3]	1960	1	43,000	16-18°S	177.5-179° E	–	–	nil	27°C
New Caledonia[4]	1965 (*h*) 1973 (*c,b*)	1	5,000	20°50′S-21°S	165°E-165°10′E	1,550	600	nil	10°C
South Africa[5]	1929	1	< 1,000	22°30′-29° S	–	950 to 1,500	600 to 900		
Venezuela	1960s	2?	> 600,000[7]	≈ 8° - 12° N	–	?	?	nil	≈ 15°C

[†] *b*, *c*, and *h* refer to var. *bahamensis*, *caribaea*, and *hondurensis* respectively.
[‡] 55,000 ha in Queensland and ca. 3000 in Northern Territory planted with *P. caribaea*, approximately another 17,000 ha planted with *P. caribaea* hybrids
[§] Estimated to reach 100,000 to 150,000 ha by 2010 (Zheng 1996, page 1-20)
[1] Jarbas Y. Shimizu, EMBRAPA-Florestas, Colombo, Brazil
[2] Wan Huoran, Chinese Academy of Forestry, China
[3] N.W. Yalimaitoga, Research & Development Manager, Fiji Pine Limited, Lautoka, Fiji
[4] Jean-Micheal Sarrailh, Manager CIRAD-Forêt, Noumea, New Caledonia
[5] Luckhoff (1964)
[6] Barrett (1991)
[7] MARNR-SEFORVEN (1997)

Table 2: Summary of the silviculture and products of *P. caribaea* Mor. by country (where available).. Information derived from survey results where indicated; otherwise, based on other information available to the authors.

Country	Rotation Length (yrs)	Important Diseases	Important Pests	Wood Specific Gravity	Main Products	Destination of products
Australia	25	nil	nil	0.45 – 0.55	Sawn timber, MDF, chip	Australia and export
Brazil[1]	12 – 25	nil	leaf cutting ants	0.40	Veneer, plywood, pulpwood	Brazil and export
China[2]	15 – 20	*Macrophomina, Denspora, Laplodermium* sp.	tip moth	0.47 – 0.58	Pulpwood, sawn timber, resin	China
Fiji[3]	20	nil	nil	0.66	Sawn timber, chips	Fiji and export
New Caledonia[4]	25 – 30	nil	nil	–	Treated posts, sawn timber	New Caledonia

[1,2,3,4] Source of information the same as in Table 1.

Table 3: Summary of current international breeding activity with *P. caribaea*. [Symbols: + , (+), ? and – refer to "yes", "possibly", "unknown", and "not applicable".]

Organisation/	Relative importance			Hybrids	Constraints to Genetic Improvement					Coop.†	Active Breeding
Country	*hond.*	*car.*	*bah.*	of interest	Wind	Seed	Pest/ Disease	Staff	Other	Collab.	Program 1997‡
Argentina-INTA	2	1	?	+	–	+	?	?	?	?	?
Australia (Qld.)	1	2	3	+	+	–	–	–	Funds	+	+
Brazil-Klabin	–	–	–	+	–	+	Ants	?	?	+	?
Brazil-EMBRAPA[4]	1	3	4	+	+	+	Ants	–	Yes – not specified	+	?
China[3]	4	1	2	+	(+)	+	+	(+)	Funds, Cold	+	(+)
CIRAD/Congo	1	–	–	(+)	–	–	?	?	?	+	(+)
Cuba	?	1	?	(+)	+	–	?	?	Funds	–	(+)
Fiji[2]	1	?	–	+	+	–	–	+	Fire	+	(+)
India	1	–	–	(+)	?	?	?	?	?	?	?
New Caledonia[1]	1	–	–	+	+	+	–	+	Small program	+	(+)
South Africa	1	–	?	+	–	+	–	(+)	Wood quality (density, resin)	+	(+)
Venezuela	1	–	–	+	–	+	?	?	?	+	?

[1, 2, 3, 4] Source of information the same as in Table 1.

† Involved in a cooperative breeding program or inter-agency collaborative projects

‡ Based solely on a subjective assessment by the authors who define "active breeding" as undertaking recurrent selection or hybrid breeding, not just establishment of one-off seed sources.

many of the stands and tests established in the 1970s are now nearing rotation age, therefore the breeder must face the question of whether of not she can afford not to conserve the genetic resources within the control of her organisation. Given the current economic imperatives, it seems likely that individual organisations will choose to rationalise theirgenetic resources of *P. caribaea*. The international (collective) consequences of these individual decisions could be very serious, perhaps disastrous, in the long term. Dvorak (1996) identifies a lack of resolve to work together as the greatest threat to advances in the exploration, conservation and utilisation of genetic resources.

HYBRIDISATION

The commercial deployment of *P. caribaea* in some environments is limited by a number of factors including: susceptibility to wind-damage, tip moth attack, concerns about wood properties, poor tolerance of periodic water-logging, and low frost tolerance. Hybridisation offers the potential to expand the potential area over which *P. caribaea* and its derivatives may be successfully deployed, through broaden adaptability, complementary combination of economically important traits, and the potential to breed for improved hybrid performance.

Potential gains through operational deployment of hybrids: *P. caribaea* produces fertile intra-specific hybrids between provenances and varieties and inter-specific hybrids with *P. elliottii*, *P. oocarpa* and *P. tecunumanii* as well as some other species (Slee 1971, Nikles 1989, Nikles 1991, Nikles 1995). Inter-provenance hybrids in var. *hondurensis* may provide the opportunity to rapidly infuse the greater wind-firmness of the coastal and island sources into the more advanced upland sources. In a test in south Queensland, an inter-provenance hybrid (Belize Mountain Pine Ridge by Coastal provenances, MPR × COA) grew at least as fast as crosses amongst the MPR provenance to four years of age, but suffered less wind damage (Figure 2). Likewise, the three varieties of *P. caribaea* display a number of complementary traits that might be combined advantageously through the use of inter-variety hybrids (Nikles 1995).

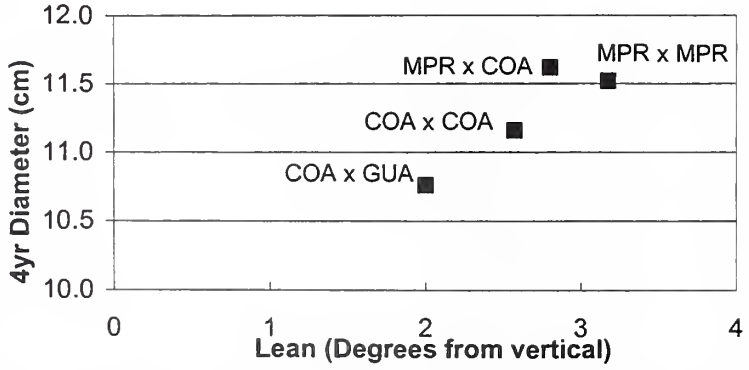

Figure 2: Wind-firmness (as measured by tree lean following strong winds) of *P. caribaea* var. *hondurensis* inter-provenance hybrids on a poorly-drained site in south-east Queensland (lat. 26° S). COA, GUA and MPR refer to coastal, Guanaja and Belize Mountain Pine Ridge provenances.

Hybrids between var. *hondurensis* and var. *caribaea* are showing considerable promise in central Queensland coastal lowlands, exhibiting very good stem straightness, fine (and flat)

branching, combined with growth rates comparable to var. *hondurensis* (Figure 3). This hybrid has also demonstrated improved wind-firmness compared to var. *hondurensis*, and hence has considerable potential in the low elevation tropics where wind-damage is a problem (e.g. Fiji, New Caledonia, northern Australia, and areas of southern China). Similarly, hybrids between var. *hondurensis* and the other two varieties are likely to combine the good growth rates of var. *hondurensis* with the resistance to tip moth. However, the insect resistance of the hybrids would need to be evaluated prior to operational deployment since Huber et al. (1997, page 14) report that *P. taeda* × *P. elliottii* hybrids inherit the susceptibility of *P. taeda* to tip moth attack, rather than the resistance of slash pine.

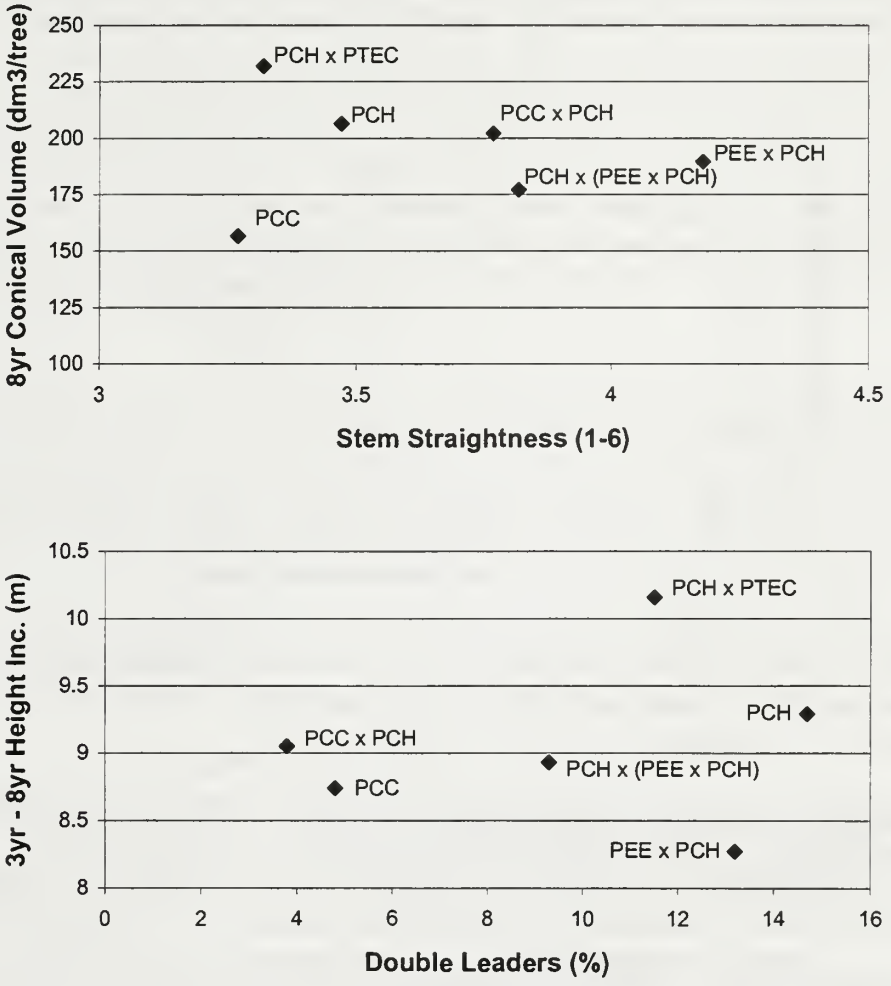

Figure 3: Volume and stem straightness, and percentage of double leaders verses tree height of *P. caribaea* var. *hondurensis* (PCH), var. *caribaea* (PCC), inter-variety hybrids, and PCH × *P. tecunumanii* hybrids at 8 years of age on a well-drained in central Queensland (lat 23° S).

Inter-specific hybrids, particularly the slash × var. *hondurensis* hybrids, are performing well in Argentina, Australia, Brazil, China, South Africa and the United States (Kikuti et al. 1996, Nikles 1991, Nikles 1995, Nikles 1996, Powell and Nikles 1996, Rockwood et al. 1991, Rockwood and Nikles 1996, White et al. 1996). The F_1, F_2 and backcross hybrids are

showing improved growth rates and branch quality compared to slash pine, and improved wind-firmness, stem straightness and wood density when compared to *P. caribaea* (Refer to Figure 4, for an example from south-east Queensland). The backcross to slash pine is likely to find application on particularly poorly drained sites, or sites subject to frosts.

Hybrids between *P. caribaea* and *P. oocarpa/P. tecunumanii* can be produced more easily than hybrids between *P. caribaea* and slash pine (Nikles 1989). Further Nikles (1989) reports that hybrids between *P. caribaea* and *P. tecunumanii* were superior in growth to both parental species across a range of sites in northern Australia and Fiji. Although these hybrids have demonstrated good growth potential (refer Figure 3), they are still susceptible to stem breakage on some sites. Therefore, the operational deployment of this hybrid will probably be restricted to sites that are not affected by strong winds, such as in eastern Venezuela.

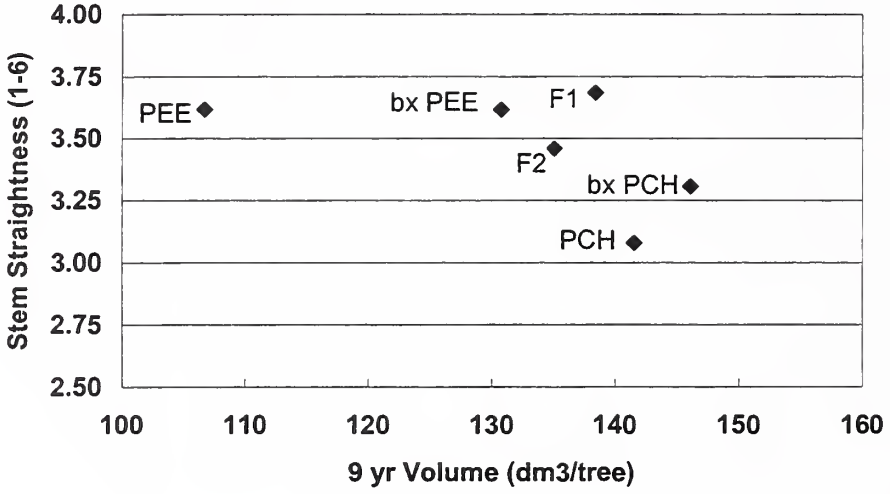

Figure 4: Volume and stem straightness (1-6, 6 good) at 9 years of age on a well-drained site in south-east Queensland (lat. 26°S) for slash pine (PEE), *P. caribaea* var. *hondurensis* (PCH) and their F_1, F_2 and backcross (bx PEE, bx PCH) hybrids.

Application of hybridisation to breeding: Hybridisation offers the opportunity to infuse genes of interest into existing breeding populations, and to obtain hybrid vigour. Most advanced breeding populations of *P. caribaea* are based on upland sources. Consequently, separate populations can be maintained for conservation purposed, but rather than maintain separate breeding programs of upland, coastal and island sources of var. *hondurensis* (e.g. Kanowski and Nikles 1988), the best coastal and island material can also be intercrossed with the best upland material. Selections in the off-spring of these crosses then carried forward into subsequent generations. If pollen from the coastal sources is used, the 'new' genes from coastal/island sources can be rapidly infused into the main breeding population at a relatively low cost.

The use of composite intra- and inter-specific hybrids to infuse favourable genes into advanced populations of var. *hondurensis* offers a number of advantages. For example:

- Only one breeding population is required, and overall breeding costs are reduced.

- Seed production problems in the first generation are over come. Seed set of the slash × var. *hondurensis* hybrid improves from approximately 20 seeds per cone in the F_1 generation to over 80 seeds per cone in the F_2. Thus, the production of large quantities of seed for operational use can be much easier.
- Favourable genes are likely to be incorporated into operational plantations much more rapidly, particularly if one population is at a considerably earlier stage of genetic improvement.

FUTURE CHALLENGES

A significant future role of *P. caribaea* in plantation forestry seems assured, both as a pure species (e.g. Venezuela) and as a parent of hybrids (e.g. Australia). Nevertheless, the future conservation status of its genetic resources is not as certain. Successive waves of introductions (especially of var. *hondurensis*) to many countries over a fairly long period, facilitated by the separate comprehensive collection and wide-spread distribution of seed by OFI and CAMCORE especially, have resulted in the *ex situ* assembly of a very large sample of the genetic resources of *P. caribaea*. The world-wide distribution of germplasm *ex situ* through a broad range of geographic regions (Table 4), has now resulted in the formation of what are effectively multiple populations, and the development of locally adapted land races. This is particularly true for var. *hondurensis*.

These regional gene pools are potentially of great significance for the long-term genetic conservation of this species, especially since *in situ* conservation seems unlikely to be a reliable long-term option, and because different adaptations are likely to have developed *ex situ*. If maintained, these *ex situ* genetic resources are likely to act as huge reservoirs of genetic variability. Currently, the genetic resources of this species are controlled by a range of government and private organisations with varying resources, and differing levels of interest in the continued conservation of *P. caribaea*'s genetic resources. Clearly, there is a need for a coordinated approach to the genetic conservation of genetic resources of this (and other widely disseminated) species.

We suggest that the following steps are necessary:

1. Documentation of the genetic resources currently held *ex situ*, perhaps using a similar system to that being tested by DANIDA/FAO (Hansen 1996). In some cases the documentation is fairly recent and accurate (e.g. CAMCORE, Queensland). This work could perhaps be sponsored by the International Plant Genetic Resources Institute (IPGRI, previously IBPGR).
2. Description of the genetic resources held in conservation stands, seed orchards and clones banks.
3. Development of a cooperative/collaborative approach to the world-wide conservation of the significant, viable holdings within each region.
4. Coordinated analyses of later-age data from the various international provenance and family-in-provenance trials of *P. caribaea*, in a similar manner to those on var. *hondurensis* by Crockford et al. (1990) and recently completed by CAMCORE (Gary Hodge, *pers. comm.* 1997) for var. *hondurensis* and *P. tecunumanii* (CAMCORE 1996).

5. Form a voluntary organisation to help coordinate this work; perhaps a separate IURFO working group dealing with *P. caribaea* would be an appropriate forum.

Table 4: The broad geographic distribution of significant *ex situ* gene pools of *P. caribaea*, and special adaptations likely to have been developed within local land races.

Geographic Region	Countries/Organisations Holding Significant Genetic Resources	Main Source of Germplasm	Variety, Provenances†	Special Adaptations Expected
Caribbean	Jamaica, Puerto Rico	OFI	*h*	Tolerance to high pH, wind-firmness
Central-eastern Brazil	CCGMT	Seed merchants, OFI	*h*, *b*, *c*	Local climatic – edaphic conditions
Eastern Venezuela	PROFORCA	Seed merchants, CAMCORE	*h* (Poptun)	Low rainfall, deep sands in tropics
Equatorial Brazil	Jari Florestal	Nicaragua	*h* (Coastal, Guanaja)	Humid tropics environment
S.E. Africa	SAFCOL	OFI, CAMCORE	*h* (+ *b*, *c*)	Local climatic – edaphic conditions
S. Brazil – N. Argentina	EMBRAPA – INTA	OFI, CAMCORE	*h*, *c*	Cold hardiness
S.E. China	CAF	Cuba, OFI	*c*, (+ *b*)	Pest and wind tolerance
S.E. India	Mysore Paper Mills	OFI, Australia	*h*	Local climatic – edaphic conditions
S.W. Pacific	Australia, Fiji, New Caledonia	OFI, CIRAD	*h* (+ *c*, *b*)	Wind-firmness, tolerance of poor soils and drainage (Aust.)
Thailand	RFD	OFI	*h*	Local climatic – edaphic conditions
W. Africa	Congo, Nigeria	OFI, CIRAD	*h*	Local climatic – edaphic conditions

† *b*, *c*, *h* refer to the *bahamensis*, *caribaea* and *hondurensis* varieties of *P. caribaea*.

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THE IMPROVEMENT AND BREEDING OF *PINUS PATULA*

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Abstract:--*Pinus patula* has a north to south geographic distribution in Mexico of approximately 900 km. It is the most commonly planted commercial softwood in southern and eastern Africa and Colombia and is characterized by its good growth, straight stem form, and excellent pulping and sawtimber properties. Nearly 1 million hectares of *P. patula* plantations are now established. The species exhibits great provenance variation. A 37% difference in volume was found between the best and worst sources at 5 to 8 years of age when grown in Brazil, Colombia, and South Africa. Provenance x site and genotype x site interaction appears to be more important in *P. patula* than in other tropical and subtropical pine species. The traits of height, volume, and stem straightness appear to be under moderate additive genetic control with individual tree h^2 of 0.27, 0.26, and 0.12, respectively, at age 8 years. Researchers have found significant juvenile-mature correlations in *P. patula* and conclude that early selection for good volume and high wood density is feasible in some environments.

Breeding programs began for *P. patula* in southern Africa in the 1950s and in Latin America in the 1970s. The most advanced programs are making crosses to begin their 3rd generation of breeding. Breeding initiatives most commonly used to develop *P. patula* are multiple breeding population strategies and an assortment of hierarchical population approaches. Estimated and realized gains for volume per generation for the first two breeding cycles are on the order of 18%. *P. patula* breeding programs should make great progress in the near future because of a) the infusion of 500 open-pollinated families from new collections in Mexico from CAMCORE, b) the use of more sophisticated statistical analytical tools for family and individual tree selection, and c) the development of vegetative propagation technology. Impediments to progress include poor seed production from controlled crosses, and low cone and seed set in seed orchards in some countries.

Keywords: pine hybrids, multiple breeding population strategy, heritability

INTRODUCTION

Pinus patula Schiede & Deppe in Schl. & Cham. is an important closed-cone pine that is native to Mexico and belongs to the same taxonomic group as *P. radiata* D. Don and *P. oocarpa* Schiede. It occurs in eastern and southern parts of the country in the Sierra Madre Oriental and the Sierra Madre del Sur between 24° N and 16° N latitude, a distance of approximately 900 km (Figure 1). Most provenances of *P. patula* are found in cloud forest environments on well drained soils between 1500 m and 3100 m altitude (Dvorak and Donahue 1992) but is most

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common at elevations of 2100 m to 2800 m (Perry 1991). *Pinus patula* occurs in regions that receive between 1000 mm and 2000 mm of precipitation annually with a distinct dry season of nearly four months. With the possible exception of provenances from southern Oaxaca, *P. patula* can withstand subfreezing weather and snow.

Pinus patula trees reach heights of 35 m and have a very distinct morphology that allows for easy identification in the field. The species is characterized by its very straight stem form, thick furrowed bark at the base of the tree, thin reddish flaky bark on most of the upper trunk, and pale-green pendent foliage. Locals refer to the species as "sad pine" or "weeping pine" because of the distinct droopiness of the needles. *Pinus patula* var. *longepedunculata*, a variety that occurs in southern Mexico (Guerrero and Oaxaca), differs morphologically from typical *P. patula* in that the cones have short peduncles and the color of the foliage may vary from pale to dark green with needles that droop less noticeably.

Pinus patula has been established in pilot plantings in as many as 20 different countries in the tropics and subtropics in the last 120 years (Wormald 1975). The first reported introduction of the species was into New Zealand in the late 1870s, South Africa in 1907, Australia in the 1920s, most other areas of southern and eastern Africa from the 1920s and 1940s, and in South America in the 1950s and 1960s (see Wormald 1975). The initial introductions into New Zealand and South Africa came from Mexico but several of the subsequent seed distributions to other countries came from existing plantations established primarily in South Africa. Interestingly, much of the genetic base for *Pinus patula* in South Africa during this period was thought to have come from only three small commercial collections in Hidalgo and northern Oaxaca (Burger 1975, Wormald 1975).

It is estimated that there are now nearly 1 million hectares of *P. patula* established in the tropics and subtropics for sawtimber and paper products (Wright 1994, Birks and Barnes 1991). The majority of the *P. patula* forests are located in southern and eastern Africa where it is the most important softwood plantation species. South Africa, Swaziland and Zimbabwe have nearly 400,000 hectares under operational management at the present time (SA Dept. of Envn. Affairs 1993, Kanzler 1997). In Colombia, 50,000 hectares of *P. patula* have been planted with lesser amounts in Argentina, Brazil, and Ecuador.

The wood of *Pinus patula* is white to yellowish-white and is moderately dense. Mature trees 30 to 50 years of age in natural stands in Mexico had wood densities that ranged from 440 to 600 kg/m^3 (Zobel 1965, Quioñes 1974). Plantation trees 13 to 16 years of age in Brazil, Colombia, and South Africa had mean wood density of 389 kg/m^3, 410 kg/m^3 and 395 kg/m^3, respectively (Wright 1994, Ladrach 1984). *Pinus patula* had wood density slightly higher than loblolly pine at 14 years of age near Lages, Brazil, (Mendes 1989) but was no more dense that loblolly pine in Chile and South Africa (Dommise 1994, Rodriguez and Torres 1992). *Pinus patula* has longer fiber length than loblolly pine (Wright and Sluis-Cremer 1992, Dommise 1994, Rodriguez and Torres 1992) and the wood has only 1 to 3% extractives, much lower than either loblolly or slash pine (*Pinus elliottii* Engelm.). These beneficial features, plus the whiteness of the wood, makes *Pinus patula* especially well suited for a wide range of products (see Wright 1994).

This paper summarizes the improvement and breeding efforts underway for *P. patula* in the tropics and subtropics. The status of new introductions from Mexico, provenance testing, and *ex situ* gene conservation plantings are discussed. The breeding strategies being used by the organizations planting *P. patula* on a commercial scale are reviewed and both the opportunities and impediments for further improvement and developments are highlighted.

Figure 1. Geographic range of *Pinus patula* in Mexico. The dashed line shows the separation of provenances that are cold hardy to the north from those that are more susceptible to cold damage to the south and west.

PROVENANCE VARIATION

Provenance Collections 1959-1984

Following the original introductions of *P. patula* into southern and eastern Africa near the turn of the century, there have been several provenance collection made in Mexico by various organizations to enlarge the existing genetic base of the species. Seeds from four provenances of *P. patula* were collected in Mexico by New Zealand researchers between 1959 and 1962 as part of a larger introduction effort to determine if Mexican pine populations that were more productive than *P. radiata* could be identified. The four *P. patula* provenances in the collections were from the states of Hidalgo, (northern) Oaxaca, and Veracruz and therefore represented the central and southern distribution of the species' range. Provenance trials were established at four locations on the North Island with the seed collected. In 1969, the South African Forestry Department made seed collections in Hidalgo, (northern) Oaxaca, and Puebla and sampled four provenances and 22 mother trees (Darrow and Coetzee 1983). Trials were established at six locations in the Eastern Transvaal. Also, in 1969, the Zimbabwe Forestry Commission received seeds from 8 provenances of *P. patula* from Mexico and planted these on 6 sites in 1971(Barnes and Mullin 1984). All provenances were from the central part of the range of *P. patula*, with the exception of one source from northern Oaxaca. The Argentineans made one of the most comprehensive collections of *P. patula* in Mexico in 1972 that included 16 provenances and 110

mother trees (Barrett 1972). This collection was unique because it was the first to include a population from the northern range of the species in the state of Tamaulipas (El Cielo 23° 05'N). The seeds were established in trials mainly in Jujuy state in northern Argentina and one at Mac-Mac, South Africa. The populations represented central Mexico. In the early 1980s, the Food and Agriculture Organization (FAO) in collaboration with the Instituto Nacional de Investigación Forestal (INIF) Mexico, distributed bulk seed lots of *P. patula* from several provenances in Mexico to interested organizations in the tropics and subtropics.

CAMCORE Range-Wide Collections and Conservation Efforts 1986-till the present

From 1986 to 1994, the Central America and Mexico Coniferous Resources Cooperative (CAMCORE), North Carolina State University, sampled 23 populations and 510 mother trees throughout the entire known geographic range of *P. patula* in Mexico (Dvorak and Donahue 1992). The objectives for the collection were to develop *ex situ* conservation field plantings, establish genetic tests, and expand the relatively small gene base of the species being managed by forestry organizations. The collections re-sampled eight of the locations visited by the Argentineans in 1972 but also included a number of new populations like the most known northern provenance, Conrado Castillo, Tamaulipas (24° N) and sources in southern Oaxaca (16° N). Seeds for 50 conservation banks and provenance/progeny tests were distributed to Brazil, Colombia, Chile, Mexico, South Africa, and Zimbabwe. The conservation banks were established in several designs ranging from single tree plots to row plots at several different spacings (Dvorak et al. 1996). The CAMCORE genetic tests were planted in a compact family design, with open-pollinated families clustered together in a provenance block. Families were planted in six-tree row plots replicated 9 times at 3m x 3m spacing.

Provenance Trial Results

In New Zealand, the provenance La Venta (near Mexico City) was the best and the population from northern Oaxaca (Llano de Las Flores) was the worst when assessed at 22 years of age for productivity and stem form. Results from the studies in Argentina (24° to 25° S latitude) indicated that the northern population, El Cielo, Tamaulipas was the most productive and the sources from northern Oaxaca were the worst (Picchi 1988). There were also several good sources from the central part of the species range in Mexico that did well in the Argentinean highlands. Results showed a strong positive correlation between latitude of the collection site in Mexico and productivity in Argentina. The results from the international CAMCORE series (Hodge and Dvorak 1998, Dvorak et al. 1996) and the local South African and Zimbabwean provenance tests (Falkenhagen 1979, Barnes and Mullin 1984) showed that most sources from the central part of the range in Puebla, Hidalgo, and Veracruz grew reasonably well. One exception to this were the provenances from the highest elevations (2750-2950 m) in both the CAMCORE and Zimbabwean tests. These sources grew slowly across most locations relative to the other provenances from central Mexico. In the CAMCORE tests, the sources from Oaxaca did poorly when planted in the colder areas of Brazil and South Africa. However, some of the southern *P. patula* sources from Oaxaca appeared to have potential when established in the tropical highlands of Colombia (Dvorak et al. 1996), Swaziland (Kanzler 1994), Zimbabwe

(Barnes and Mullin 1984), and in the warmer areas of South Africa (Hodge and Dvorak 1998). Trees from the El Cielo, Tamaulipas source in northern Mexico performed well in the CAMCORE tests when planted on sites with temperate climates. However, it and the other northern source, Conrado Castillo, performed poorly for volume across locations when established on warmer sites in the subtropics (Dvorak and Hodge 1998).

The CAMCORE international series of provenance tests demonstrate the importance of continued explorations and seed collections in the natural range of the species for both the purposes of conservation and genetic testing. Several new populations from the state of Veracruz were found that were 14% to 18% better in volume than the average of all provenances across 50 test locations. The productivity of the sources from Veracruz were within 7% of the volume performance of second generation check lots (Hodge and Dvorak 1998). The best families from these excellent Mexican sources were competitive in volume with many first and some second generation *P. patula* controls. The difference in volume between the best and the worst provenances across all locations in the CAMCORE *P. patula* series was 37% (Hodge and Dvorak 1998).

Cold tolerance, drought tolerance and disease and insect susceptibility

As alluded to before, provenances of *P. patula* from southern Oaxaca (and presumably Guerrero) that comprise the variety *longepedunculata* are not nearly as cold hardy as sources to the north. The morphology of some of these populations indicate that they may carry *P. tecunumanii* genes but appear to group separately from either typical *P. patula* or *P. tecunumanii* (Dvorak and Raymond 1991) Three year results of a number of CAMCORE tests in South Africa, where freezes can sometimes be severe, showed that the average survival of the southern Oaxacan sources to be 26% and the rest of the provenances 70% (Dvorak et al. 1996).

Pinus patula is generally found to be more drought tolerant than loblolly pine and in a recent severe drought in southern Africa was found to be as drought tolerant as slash pine (Morris and Molony 1993). The species is very wind firm as the needle architecture promotes the passage of wind through the upper crown instead of serving as a barrier to it. Wind throw is only a problem when poor nursery practices or silviculture management has promoted poor root system development or stand overstocking (Munishi and Chamshama 1994). *Pinus patula* does not perform well on sites that are often wet or have poor drainage. Furthermore, the species is very susceptible to *Sphaeropsis sapinea* (formerly called *Diplodia*) in both South Africa and Brazil. Hail is usually the casual agent that damages the trees that promotes *Sphaeropsis* infection in South Africa (Swart and Wingfield 1991) and it is hypothesized that late spring frosts that damage growing shoots may be the event that initiates the disease in Brazil (CAMCORE Annual Report 1996). *Pinus patula* susceptibility to aphid attacks in southern Africa has been well documented as is its tendency to be defoliated by *Glena* spp. in both Colombia and Brazil (Rodas 1996, Martin et al. 1994) and the tent caterpillar (*Lechriolepis nephopyropa tams)* in Zimbabwe (Mushongahande 1996). *Pinus patula* seedlings have also been screened for resistance to fusiform rust (*Cronatium quercuum* (Berk.) Miyabe ex Shirai f. sp. fusiforme) in the US and in one study was found to have a 30% infection rate versus 64% for the slash pine control (Tainter

and Anderson 1993). This is much lower infection rate than some of the other Mexican closed-cone pines like *P. caribaea* and *P. tecunumanii* (Lambeth et al. 1997). Oak species (*Quercus*), the alternate host to fusiform rust, are common throughout the natural range of *P. patula* in Mexico.

REPRODUCTIVE BIOLOGY

Observations on Flowering

Poor seed or cone crops have often been given as the reason for the limited success in making *Pinus patula* seed collections in Mexico (Darrow and Coetzee 1983, FAO 1972). However, the fact is that *Pinus patula* is often a very shy seed producer in its native environment even during good seed years.

In natural stands in Mexico, male and female strobili of *P. patula* are produced from January to April and cones are produced 22 to 24 months later (Romero 1991, Patiño and Kageyama 1991). The first crop of cones on trees in natural stands may not be produced until approximately 15 years of age (Patiño and Kageyama 1991). The cones of *P. patula* range in size from 70 to 100 mm and may be borne in pairs or in clusters of up to 5 to 6. The cones of the southern variety *longepedunculata* are smaller and range in size from 50 to 80 mm. These are often borne singularly or in pairs. The seed potential of a cone of *Pinus patula* and its variety *longepedunculata* is 125 and 95, respectively (Dvorak 1997). An average of 22 filled seeds per cone were found in studies in natural populations of *P. patula (*Barrett 1972) for a seed efficiency rate of 18%. This value is the lowest of the Mexican closed-cone pines with the exception of the high elevation populations of *P. tecunumanii* where only 6 filled seeds were found per cone; a seed efficiency rate of 9% (Dvorak and Lambeth 1992). There are approximately 115,000 seeds per kg in *Pinus patula.*

Pinus patula begins to flower at 2 to 3 years of age in many locations in southern Africa but takes longer at high elevations near the equator in Latin America. As expected, flowering times vary considerably where *Pinus patula* is planted as an exotic. In southern Colombia (2° N latitude), *Pinus patula* flowers all during the year with a peak in July and August (Isaza 1997). In southern Brazil (27° S latitude), the flowering time appears to be in September but more study is needed (Mendes 1997). In southern Africa (18 to 28° S latitude), there are two flowering periods, a small peak from January to May and a more pronounced peak from September and October (Barnes and Mullin 1974, van der Sijde and Denison 1967). Most breeding work is done on the flowers in the second semester. A bi-annual flowering period has also been observed for *Pinus greggii* in several locations where it has been planted as an exotic species (Critchfield 1967, Kietzka 1997).

Environmental Factors and Seed Production

Environmental influences have affected the reproductive biology cycle of *P. patula* when planted as an exotic and have caused delays in seed production and breeding efforts in several countries.

In both Colombia and South Africa (Denison 1973), initial attempts to establish seed orchards met with limited success because they were located at elevations too low for good seed production and had to be moved to higher altitudes. Studies now show that in Colombia an altitude of 2500 m is acceptable for a *P. patula* orchard if supplemental mass pollination is used. Supplemental mass pollination has not only increased the number of filled seeds per cone from approximately 6 to 30 but has increased by five-fold the number of cones per tree that reach maturity (Wright 1997). In southern Africa studies now show that the optimum altitude for a *Pinus patula* seed orchard appears to be 1900 m in the eastern highlands of Zimbabwe (19°S), 1500 m in the Transvaal, South Africa (25°), and 1450 m in Natal, South Africa (29°S). A mean annual temperature between 13° and 16° C was considered best for cone and seed production by Barnes and Mullin (1974). When seed orchards are properly located in southern Africa, 50 to 70 filled seeds per cone can be obtained.

A second factor that has affected not only breeding efforts for *P. patula* but also has influenced the type of breeding strategy adopted (discussed later) by several organizations in South Africa is the poor seed yields being obtained from artificial crosses. Results from one orchard indicated that 60 filled seeds per cone were obtained from open-pollination and 10 filled seeds per cone from controlled crosses (Kietzka 1997). *Pinus patula* flowers are much more sensitive to heat build-up in pollination bags than either loblolly or slash pine and can be easily destroyed in spring when the temperature rises rapidly during the day (van der Sijde and Denison 1967). Studies are underway to find a pollination bag that provides better heat exchange. The flower abortion problem during controlled crosses appears to be less in Zimbabwe, which has a more tropical climate but generally has less daily temperature fluctuations.

BREEDING STRATEGY

Breeding Programs

The first breeding programs for *Pinus patula* in South Africa and Zimbabwe began in the late 1950s (Barnes 1995, Denison 1973). Tree improvement activities were facilitated in the region in the 1960s and early 1970s in Kenya, Tanzania and Uganda through participation in the East African Agriculture and Forestry Research (EAAFRO) that allowed for an interchange of *P. patula* scion material across country (Dyson 1977). Today, most countries in southern Africa involved in plantation forestry have at least a first generation *P. patula* seed orchards made up of clones selected throughout the region. In Latin America, only Smurfit Cartón de Colombia in Cali has an active breeding program for *Pinus patula* and this began in the early 1970s.

Some of the *P. patula* programs are well into their second generation of breeding and now have third generation material. Five of these organizations have been highlighted for the purposes of discussing breeding strategies: Mondi Paper Co., the South African Forestry Company (SAFCOL), South Africa, SAPPI (Pty) Ltd., South Africa, Smurfit Cartón de Colombia, and the Forestry Research Centre, Zimbabwe. All are participating in the CAMCORE program in efforts to broaden their genetic base of *P. patula*. The number and origin of families in their breeding programs are summarized in Tables 1 & 2.

Table 1. Number of families in genetic tests of *Pinus patula* by generation for selected programs in South America and Africa (from Bester 1997, Gapare 1997, Kietzka 1997, Stanger 1997, Wright 1997).

Organization	Country	CAMCORE	1st Gen.	2nd Gen.	3rd Gen.
Cartón de Colombia	Colombia	435	91	37	
Mondi	South Africa	405	652	299	37
SAPPI	South Africa	468	882	136	---
SAFCOL	South Africa	366	1495	671	---
Research Centre	Zimbabwe	122	463	393	---

Table 2. Origin of plus tree selections represented in genetic tests of *Pinus patula* for breeding programs in South America and Africa.

Origin	Cartón Colombia	Mondi S. Africa	SAPPI S. Africa	SAFCOL S. Africa	Res.Centre Zimbabwe
CAMCORE	435	405	468	366	122
Colombia	41	---	---	---	---
ICFR[2]	---	154	391	146	---
Malawi	---	7	---	---	---
Mexico	---	83	---	51	---
South Africa	87	643	611	1185	70
Zimbabwe	---	101	16	42	438

Genetic Parameters

Heritability and Genetic Correlations

There have been a number of studies using both open and controlled pollinated test material to assess the magnitude of additive variance, degree of genetic correlation and importance of genotype x site interactions in *Pinus patula*. Individual tree heritability for height for *P. patula* ranged between 0.14 and 0.22 at 5 to 8 years of age in open-pollinated studies in Brazil and full-sib experiments in Zimbabwe (Kageyama et al. 1977, Barnes et al. 1992). Heritability values for diameter were lower than for height and ranged from 0.13 to 0.18 (Kageyama et al. 1977, Nyoka et al. 1994). Individual tree heritability values for height and diameter for open-pollinated trees assessed at between 5 and 8 years of age in the highlands of Colombia were as much as two times higher than those reported in Brazil and Zimbabwe (Ladrach and Lambeth 1991, Wright et al. 1996). Possibly this was due to the fact that trees in Colombia measured at 8 years of age are

[2] ICFR (Institute for Commercial Forestry Research), Pietermaritzburg, South Africa

as tall as trees in other countries measured at 10 to 11 years of age and direct comparisons of additive genetic variance across country are biased somewhat by differences in ontogeny (Vásquez and Dvorak 1996). In individual sites analyses of 50 tests in Brazil, Colombia, and South Africa using CAMCORE material, individual tree heritability at ages 5 and 8 years for volume were 0.16 and 0.26 respectively (Hodge and Dvorak 1998). The age 8 heritability value for volume was approximately the same as that found for *Pinus caribaea* var. *hondurensis* but was 0.05 higher for *Pinus tecunumanii* in similar continent-wide plantings. Heritability for stem straightness in *P. patula* at 5 to 8 years of age ranged from 0.04 to 0.32 in studies in Brazil and Zimbabwe (Kageyama et al. 1977, Barnes et al. 1992, Nyoka et al. 1994) and from 0.09 to 0.12 in the individual site analyses done on 50 locations in the CAMCORE tests (Hodge and Dvorak 1998).

In a series of controlled cross experiments in Zimbabwe, Barnes et al. (1992), found strong juvenile-mature correlations between traits measured in the nursery and characteristics measured in the field at 8 years of age. Large seedlings with few cotyledons in the nursery grew into large trees with high wood density in the field and families with few branches and superior height in the second year developed into trees with high wood density and large volume the eighth year. Type B genetic correlations among open-pollinated families in CAMCORE tests in Brazil, Colombia and South Africa were sufficiently high to be useful in best linear prediction (Hodge and Dvorak 1998). Genetic information from families grown in one country could be used to improve prediction of performance in another country.

Provenance x Site and Genotype x Site Interaction

Because trees from the southern Oaxacan provenances suffered freeze damage on the more temperate sites but survived well on the more tropical sites, provenance performance for height and volume was generally unstable across wide locations in the CAMCORE tests. In addition, there appears to be more provenance x site interaction for populations from the central part of the *P. patula* range when grown across exotic environments than for either *P. caribaea* or *P. tecunumanii* (Hodge and Dvorak 1998). Height/diameter ratios were found to change noticeably across environments (Denison 1973, Dvorak et al. 1996). Important genotype x site interaction for volume at the family level was found across location in the international series of CAMCORE tests as well as those trials established on several sites in separate studies in both South Africa and Zimbabwe (Barnes et al. 1992; Falkenhagen 1979). Hodge and Dvorak (1998) suggest that genotype x site interaction may be greater in *P. patula* than in *P. caribaea* var. *hondurensis* or *P. tecunumanii*. Genotype x year interaction does not appear to be important in *P. patula* (Barnes et al. 1992).

Breeding strategies and mating designs

A number of breeding strategies and mating designs have been used to improve *Pinus patula* in the last 40 years. Several organizations in southern Africa are now using a multiple breeding population strategy (MBPS) that was first proposed by Namkoong et al. (1980) and later refined by Barnes (1994). The strategy allows one to work with a number of populations at different level of intensity with the goal to turn over generations as quickly as possible. The MPBS was

developed for those organizations or countries in the tropics and subtropics that were working with many species and provenances, were not part of a cooperative breeding program, and had limited research staff or funds. The mechanism by which the MBPS is carried out in South Africa and Zimbawe is the breeding seed orchard (BSO), which, for all practical purposes, can be considered a combination seedling seed orchard/ progeny test (Barnes 1995).

As a practical example, the development of the MBPS by SAPPI (Pty) Ltd. (South Africa) has been described as follows by Stanger (1997). SAPPI has identified five different combinations of land types and/or elevation gradients in its plantations at Mpumalanga that it has separated into different breeding populations. In addition, it has identified selections from geographically different areas like Zimbabwe and Mexico as unique to the Mpumalanga material and these have also been placed into separate breeding populations. The arrangement of the 384 open-pollinated families in the 8 MBP are shown in Table 3. The breeding populations have been established in BSO's with five standard checks. All families have been established in 12 tree row plots with 10 replications at a spacing of 3.0 m x 1.5 m. The reason for the close initial spacing is to increase the selection intensity. The family plots will receive successive 50% thinnings at the onset of competition, the first thinning usually occurs at approximately 30 months. Before the first thinning the BSO will be assessed and the data analyzed to obtain family and within family information. After the first thinning no further measurements are usually made until the final tree per plot remains. A BSO may receive as many as five thinnings before reaching a final stocking of approximately 185 stems per hectare without family roguing. Open-pollinated seeds will be collected from the best trees in the best families using a selection index and the 45 to 50 trees selected will be used to establish the next generation of BSO's. A family culling of approximately 50% will also be done to remove the worst families and to convert the BSO into a seedling seed orchard for commercial production. At the same time that seed is collected from the best 45 to 50 trees, scion material is collected and grafted into a clone bank/orchard that will be culled when results from the next generation of tests become available. If the flowering and cone production is acceptable, a new generation can be established with open-pollinated seed every eight years. No estimates of gains have been made with the MBPS approach since the BSO's at SAPPI are still young.

Table 3. Composition of SAPPI (South Africa) Eight Breeding Populations (from Stanger 1997)

Population Origin	Type	No. of Families	Year Established
Mpumalanga	Land Type 601	42	1992
Mpumalanga	Land Type 507	41	1993
Mpumalanga	Land Type 405-1	46	1994
Mpumalanga	Land Type 405-2	47	1995
Mpumalanga	Land Type 312	60	1996
Mexico	Veracruz	35	1995
Mexico	Non-Veracruz	53	1993
Zimbabwe	------	60	1992

Other organizations that are breeding *P. patula* are using either the MBPS approach or a combination of hierarchical strategies that incorporate sublines, main and elite populations etc. to further improve the species. These programs are briefly summarized below.

a) Mondi, South Africa has structured its sublines based on origin of the material. The main population is generated by open pollinations and the elite population by controlled crosses. The present testing design is a randomized complete block with five replications and 6 tree row plots. This will be amended to single tree plots in the near future. Current selections are being made using BLP analysis. Realized gains for volume in the 1st generation was 20% and it is estimated that 2nd generation material will be 15% better than 1st generation planting stock (Kietzka 1997).

b) SAFCOL, South Africa has established one main breeding population for *P. patula* in its most important planting area for the species in the Transvaal. An open-pollinated approach was primarily used between first and second generation and currently second generation selections are being mated in a half diallel scheme. The controlled crossing program should be finished in 1997. The field tests were initially established using a RCB design, nine replications and six tree row plots but more recently lattice and single tree plots are being used. The selections in the tests are being made using BLP analysis. (Bester 1997).

c) The Forest Research Centre, Zimbabwe began its first generation *P. patula* program in 1958 based on the concept of hierarchy of populations and included using polycross, factorial and diallel mating designs (Barnes et al 1992). In 1981, the MBPS was adopted and 10 active populations developed. An intensive breeding program was implemented for *P. patula*, which included complete pedigree control through controlled pollination and full-sib families. To create full-sibs, a circular mating design is used where every parent is crossed with two other parents. Most recently, the MBPS has been amended. Multiple breeding populations in the future will be combined into one population called a composite breeding seed orchard (CBSO) to increase genetic gain and reduce costs (Nyoka et al. 1996). The best selections in the *P. patula* CBSO will make up the elite population for the next generation. Future selections will be made with a combined index generated by BLUP (Nyoka et al. 1996). Estimated genetic gains in volume production were 17% and 18% for the first and second generation, respectively (Wanyancha 1990).

d) Smurfit Cartón de Colombia initial selections in Colombia came from seeds of both South African and Zimbabwean origin. It has a first generation breeding orchard made up of plantation selections from this material and will probably use a complementary mating approach (polymix and partial diallel) to generate breeding material for the next generation. Open-pollinated progeny from the first generation orchard is being tested on two sites. The CAMCORE infusion material will form the majority of its genetic base and will be controlled crossed to produce superior progeny for the next cycle of breeding. Selections for the breeding population are currently based on BLP analysis (Wright 1997).

PROPAGATION AND DEPLOYMENT

Most organizations growing *Pinus patula* are planting seedlings rather than cuttings from seedling stools. However, this will change greatly in the next 10 years as propagation systems become more wide spread. Organizations in both Colombia and South Africa have developed the technology to mass produce cuttings from seedlings. Furthermore, *P. patula* was easily propagated and multiplied in Chile using the Arauco systems originally designed for *P. radiata* D. Don. (Balocchi 1996).

Smurfit Cartón de Colombia and Mondi now deploy 5-10% of all their *P. patula* as cuttings and the rest as seedlings (Wright 1997; Kietzka 1997). Furthermore about 5% of the seedlings that are established by Mondi are planted in family blocks but this will increase as enough seed becomes available by clone in the seed orchard. Mondi is using 70 clones operationally for its seedling program and 25 clones for cuttings.

DISCUSSION AND CONCLUSIONS

There have been a number of changes in *Pinus patula* breeding in the last 5 to 8 years that will greatly improve the productivity of the species. First, a large group of open-pollinated families from Mexico has been introduced by CAMCORE to enlarge the existing genetic base of most of the major *P. patula* programs in the world. Some of the best families from these collections of unimproved material compete very well in terms of volume with improved first generation families and some second generation families in existing *P. patula* programs. Second, vegetative propagation technology has been developed for the species and it will be possible to capture additional productivity and uniformity gains from the best controlled crosses. Third, more sophisticated statistical analysis are being used such as BLP or BLUP by *P. patula* growers to better identify good families and individuals. Genetic correlations in growth are sufficiently high between countries to improve breeding value estimates across breeding regions and provide the benefits of exchange of genetic material in the future. Fourth, because it can be hybridized easily with several of the other closed-cone pines like *P. greggii, P. oocarpa, P. tecunumanii,* and *P. radiata,* tremendous opportunities exist for the development of more productive forests in areas that are now marginal for *P. patula* (Lambeth at al. 1997, Dvorak et al. 1996). Fifth, a new area of *P. patula* var. *longepedunculata* has been recently identified in Guerrero, Mexico several hundred km from known populations in the eastern part of the country (Donahue 1990) Genetic material from this region may be especially promising in the more tropical and humid areas where typical *P. patula* is currently being planted.

Several challenges do remain in *Pinus patula* breeding. First, now that better knowledge exists on placement of breeding orchards everything possible must be done to reduce the generation time. The breeding cycle was as long as 15 to 20 years for some programs in the first generation for reasons described in the paper. Second, exchange of genetic material between organizations of the best *P. patula* material must become common place. Such exchanges are planned through the CAMCORE program, but benefits could also be derived from strategic exchanges of more genetically advanced non-CAMCORE material between the most active breeding organizations.

Third, disease, insect, and nutrient problems in *Pinus patula* need to be more intensively studied in some countries, particularly those in southern Latin America.

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BREEDING TROPICAL AUSTRALIAN ACACIAS

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Abstract: There are over 1200 species in the genus *Acacia*, over 900 of them in Australia. Plantations of four fast-growing species of the section *Juliflorae*, subgenus *Phyllodineae*: *Acacia mangium, A. auriculiformis, A. crassicarpa* and *A. aulacocarpa* are becoming important wood sources for paper and timber in south east Asia. These species grow naturally in northern Australia, Papua New Guinea and Irian Jaya (Indonesia).

Introductions of *A. mangium* to south east Asia began in the 1970s when seeds of a single tree collected at Mission Beach in Queensland were grown in Sabah (Malaysia). This initial introduction produced a land race of poor vigour. Earlier informal introductions of *A. auriculiformis* similarly resulted in land races of poor form and vigour. Representative provenance collections of the four species were made by CSIRO in the 1980s and tested not only in Sabah, but more widely in Indonesia, Malaysia, Thailand, Vietnam, and China. Superior provenances, mostly from Papua New Guinea, have been identified and results suggest there is relatively little provenance-by-environment interaction. Many operational plantation programs now use bulk provenance seedlots collected from the best known provenances.

Controlled pollination in these tropical *Acacia* species is very difficult and so breeding is largely based on open pollination. Trees flower and set seed within 3-4 years of age and so present the opportunity for rapid genetic improvement through quick turnover of generations. Open-pollinated breeding populations and seed orchards representing a broad genetic base of the best provenances of *A. auriculiformis* and *A. mangium* are now established in many south east Asian countries and in north Queensland, Australia, with a number of second-generation breeding populations already planted. Some countries have begun similar improvement programs with *A. aulacocarpa* and *A. crassicarpa.*

The interspecific hybrid between *A. auriculiformis* and *A. mangium* shows outstanding form and vigour in some tropical environments and, like the parent species, can be propagated from basal cuttings of young (up to 3 years old) trees. This provides an option for accelerated genetic gain through clonal forestry.

Keywords: Acacia, genetic improvement

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Acacia species native to Australia have been planted in over 70 countries and the area of plantations totalled approximately 1,750,000 ha in 1996 (Australian Tree Seed Centre estimates). Doran (1997) provides species profiles for the most promising Australian acacia species. *Acacia mearnsii*, native to temperate south eastern Australia, is widely planted in southern and central Africa and Brazil (200,000 ha), providing fuelwood, pulpwood and tannin from the bark. *Acacia saligna*, from south western Australia, is widely planted for fuelwood and animal fodder in north Africa and the Middle East. *Acacia colei* and *A. holosericea* from the subtropical dry zone are planted in semi-arid regions of Sahelian Africa and India for fuelwood, and their edible seeds are now being evaluated as a source of human food (Harwood 1994, Thomson *et al.* 1994).

The largest areas of *Acacia* plantations comprise two species from the tropical humid/subhumid zone, *A. mangium* and *A. auriculiformis*, now widely planted in lowland tropical and subtropical south east and southern Asia and southern China. (Table 1). *Acacia crassicarpa* and to a lesser extent *A. aulacocarpa* are also becoming important plantation species in these regions. The area of plantations is currently expanding very rapidly, particularly in Indonesia, and is expected to more than double by the year 2010. *A. mangium* has been found to be one of the tree species most effective for afforestation of *Imperata* grasslands which cover an estimated 20 million hectares in south east Asia (Turvey 1994). Some plantation estates such as that of SAFODA in northern Sabah, Malaysia, have established *A. mangium* plantations primarily on *Imperata* grasslands, but many other operations are planting on cut-over tropical forest.

Table 1. Plantation areas in 1996 of tropical *Acacia* species in south east Asia (primarily *A. mangium* and *A. auriculiformis*) (Source: ATSC estimates).

Country	1996 plantation area (ha)	Anticipated plantation area 2010
China	70,000	150,000
Indonesia	500,000	1,800,000
Malaysia	91,500	980,000
Papua New Guinea	10,000	?10,000
Philippines	45,000	?45,000
Thailand	20,000	50,000
Vietnam	80,000	150,000
Total	**815,500**	**>3,185,000**

Acacias are nitrogen-fixing members of the family *Leguminoseae*, sub-family *Mimosoideae*, and there are over 1200 species in the genus world-wide, more than 700 native to Australia. (Pedley 1981). The taxonomy of such a large genus is dynamic and there have been attempts

recently to split it into three new genera (*Acacia, Senegalia* and *Racosperma,* Pedley 1987). Of these three groups of species one (*Acacia*) has species with bipinnate-leaves and spiny leaf axils growing mostly in Africa, Asia, South America, the second (*Aculeiferum*) consists of prickly vines or small trees widely distributed in the tropics, and the third (*Heterophyllum*) is mostly restricted to Australia and nearby islands. *Heterophyllum* contains both non-spiny, bipinnate-leaved (*Botrycephalae* and *Pulchellae*) and phyllodinous (where "leaves" are flattened stems) species (Pedley 1981).

This paper reviews the genetic improvement of *Acacia* species, focusing primarily on the tropical species *A. mangium*, *A. auriculiformis, A. crassicarpa* and *A. aulacocarpa* which have recently attained importance as plantation species. These are phyllodinous species belonging to the section *Juliflorae* in the *Heterophyllum* subgenus.

BIOLOGICAL CHARACTERISTICS

Australian acacia species, like most legumes, fix nitrogen through a symbiotic relationship with *Rhizobium* bacteria. This makes acacias useful in increasing organic matter without affecting sometimes precious nitrogen resources in the soil. Some strains of *Rhizobium* are more effective than others, and inoculation with superior strains in the nursery has been demonstrated to produce significant increases in biomass production of *A. mangium* in the field, in some exotic environments (Dart *et al* 1991, Galiana and Prin 1996). However, little attention is paid to *Rhizobium* strains in the southeast Asian countries where acacias are planted most widely, because nodulation occurs naturally in nurseries using forest topsoil in the potting mix, without artificial inoculation.

Flowering. In favourable environments, flowering of *A. mangium* occurs when trees are only 1.5-3 years old. *A. auriculiformis*, *A. aulacocarpa and A. crassicarpa* tend to be slightly less precocious but all will set seed within 3 years under the most favourable conditions. In the natural range in north Queensland, flowering of these species is seasonal, occurring mainly between March and May. Individual trees typically have several flowering flushes over this period. Seeds are mature by October-November. At equatorial latitudes in Malaysia, flowering and fruiting occur sporadically throughout the year, but usually with one or more major flushes (Zakaria 1993).

The inflorescence of *Juliflorae* acacias is a spike formed of many flowers (Sedgley 1987). Pollen grains are grouped into polyads (Kenrick and Knox 1982) consisting of 16 grains (4, 8, 12, 16, 32, 64 in other spp). Flowers are mostly hermaphroditic (only 4% staminate, Zakaria 1993), but although other acacias are protogynous (Sedgley 1987), Zakaria (1993) reports that in *A. mangium* there is synchronous emergence of stigmas and stamens with stigmas becoming receptive after stamen dehiscence. Other reports (eg Sedgley and Harbard 1993) suggest that this species too is protogynous.

16-grained polyads are formed from a single sporogenous cell by two mitoses followed by a single meiotic division (Kenrick and Knox 1979). When stigmas are pollinated by a single polyad, all resulting seeds have the same male parent although there are still differences

among the seeds, the products of different meioses. In studies of *A. melanoxylon* (a related species with 16-grained polyads), Muona *et al.* (1991) found that a small but varying proportion (8-15%) of seed pods contained seeds derived from two or more male parents. They also found that different pods within the same inflorescence were more likely to have the same male parent than pods from different inflorescences. If these findings apply to the tropical species under consideration here, it has considerable implications for estimates of genetic parameters and for breeding strategy. When collecting seeds for progeny tests, too small a sample of pods and pods from the same inflorescences will lead to very high full-sibbing rates among progeny. To capture a high level of genetic diversity in seed collections it is necessary to collect many pods from different parts of the crown of each tree.

Controlled pollination techniques have been developed for acacias (Sedgley *et al.* 1992a, Jiwarawat *et al.* 1996) but have yet to become adopted widely. The number of control-pollinated seeds which can reliably be produced by a skilled worker is low - only 100-200 or so per week of full-time work. This would make it very expensive to regenerate a large breeding population through controlled pollination.

Mating system. Most acacias seem to have high rates of outcrossing (Sedgley 1987). Multilocus estimates for two natural populations each of *A. crassicarpa* and *A. auriculiformis* suggested extremely high outcrossing rates with little variation among populations (Moran *et al.* 1989a). This suggests that deviations from half-sibbing among progeny resulting from open-pollination will be derived from pollination by the same non-self male parent (via polyads) rather than from self-pollination. *A. aulacocarpa*, closely related to *A. crassicarpa* also had very high outcrossing rates (McGranahan *et al.* 1997). Further isozyme studies on 7 natural populations of *A. auriculiformis* provided estimates of multilocus outcrossing rates ranging from 0.67 to 0.95, the lowest being from a population in the Northern Territory which had low genetic diversity (Wickneswari and Norwati 1995). Reliable estimates of outcrossing have not been obtained for *A. mangium* because it exhibits extremely low levels of heterozygosity at isozyme loci (Moran *et al.* 1989b). Recent studies using RFLP markers (Butcher *et al.* 1996) have revealed higher levels of genetic variation in this species and outcrossing estimates could be made using these markers.

The most important pollination vectors for the tropical acacias under review here are believed to be insects, particularly bees which carry pollen polyads trapped on their hairy bodies (Sedgley *et al.* 1992).

HYBRIDS.

The best-known hybrid among the tropical acacias is that between *A. mangium* and *A. auriculiformis*. It was first recognised in Sabah in 1972 growing amongst roadside planted trees (Pinso and Nasi 1991). It is thought that it originated from planted trees of *A. mangium* crossing with nearby wildlings of *A. auriculiformis* introduced many years earlier (Mr Shim Phiau Soon pers. comm. 1992). Some hybrid individuals have shown outstanding growth in some environments (eg Sabah, Lapongan 1987, and Vietnam, Kha 1996). Its morphological

characteristics (eg phyllode shape, size and venation, Kha 1996, and pod shape) seem to be intermediate between the two parent species but there appear to be more staminate flowers, particularly at the base of flowering spikes (Kijkar 1993). Interest in the hybrid has led to the establishment of biclonal seed orchards of the two species in Sabah (Griffin *et al.* 1991). It is relatively simple to identify hybrid seedlings in the progeny of either species (Gan and Sim 1991). Options for vegetative propagation are similar to those of the parent species (see below). Although not necessarily a disadvantage, the hybrid has very low seed set. Progressive decline in growth rates observed in successive generations of plantings in Sabah (Sim 1984) could have been due to hybrid breakdown (Mr Shim Phiau Soon pers. comm. 1992) as much as inbreeding depression (see below).

Vegetative propagation

It is easy to propagate young seedlings of these three species by stem cuttings, but the ability to form roots drops rapidly after 12 months of age. Darus (1991, 1993) obtained 71% success with 6 month seedlings of *A. mangium*, but by 12 months, this had fallen to 31% and by 24 months was only 15%. Similar results for *A. mangium* were obtained by Monteuuis (1995). Coppicing of 3-year-old selections of the *A. mangium* x *A. auriculiformis* hybrid in Vietnam and cutting propagation of resprout shoots enabled 21 of some 60 selections to be brought into large scale propagation for clonal testing, other selections had to be abandoned because they rooted poorly.

Coppicing is not vigorous or reliable, so these species cannot be grown on coppicing rotations.

High success rates (70%) have been obtained by using marcots on adult trees (Sim 1987). Marcotting does not fully restore juvenility and rooting ability. Young shoots from some marcots can be used as cuttings to produce ramets of a clone (Wong 1988), but rooting vigour of such cuttings is not good enough for operational clonal forestry.

Propagation of juvenile material is also possible by micropropagation; Darus (1991) used nodal explants to obtain cultures from 1-month-old germinants. He claimed it was possible to obtain shoots after 2 weeks and multiplication rates of about 20-30 per explant, but in his experiments the rooting percentage fell from 60-70% in initial cycles to 50% at cycle 6 (Darus 1993). This drop in rooting percentage with repeated cycles of cuttings in tissue culture was not matched in studies of cutting propagation reported by Wong and Haines (1992) in *A. mangium* and *A. auriculiformis*. Haines and Griffin (1992) suggest that stem cuttings are adequate for vegetative propagation of these tropical acacias and the hybrid *A. mangium* x *A. auriculiformis* that tissue culture offers insufficient advantages to warrant wide adoption. However, tissue culture does have the capacity to achieve at least partial rejuvenation of selected genotypes by culturing axillary buds of trees several years old, in the case of the *A. mangium* x *A. auriculiformis* hybrid (Kha 1996), although it is unclear to what extent it can restore good rooting ability in hard-to-root genotypes.

DISTRIBUTION

Acacia mangium, A. auriculiformis and *A. crassicarpa* all occur naturally in coastal sub-coastal north east Queensland, south west Papua New Guinea and adjacent south east Irian Jaya (Pinyopusarerk 1990, Thomson 1994, Butcher *et al.* 1996). *A. mangium* also occurs on the Indonesian islands of Ceram and Aru and in northwestern Irian Jaya. *A. auriculiformis* occurs in Australia's Northern Territory and in some Indonesian islands off Irian Jaya as well as Queensland and PNG. *A. aulacocarpa* occupies a more extensive range including north western Western Australia, Northern Territory, Papua New Guinea and Indonesia, and its range extends south along the Queensland coast as far as New South Wales. The taxonomy of *A. aulacocarpa* is currently under revision and the populations with good potential for plantation forestry are restricted to southern New Guinea and north eastern Queensland (Thomson 1994).

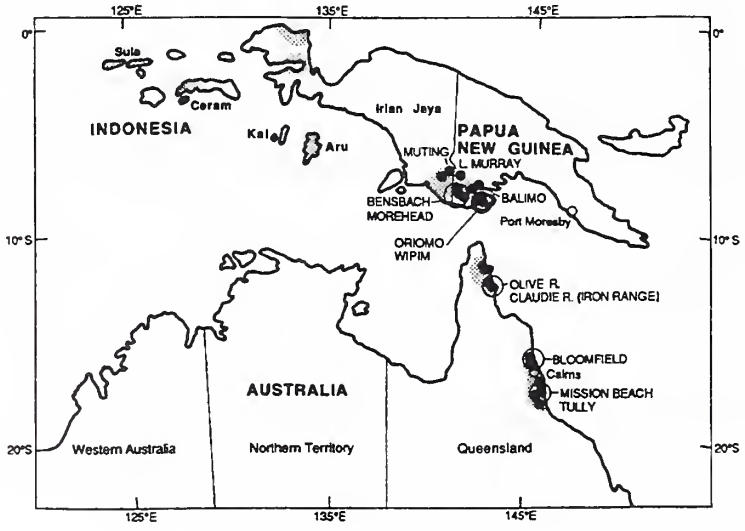

Fig 1. Map of the natural distribution of *Acacia mangium*

EXPERIMENTAL RESULTS

A. mangium grows extremely fast in the humid and seasonally dry tropics, producing up to 7.2m/y in height growth (14.4m in 2 years, Racz and Zakaria 1987), but more generally over 4m/y for the first 2-4 years, dropping to 2-2.5m/y after that (Lim 1987). MAI for diameter varies from 1.8cm/y to 7.4 cm/y. Many published volume production estimates are available from young research plantings (eg. Sim 1987, Turvey 1995, Otsamo *et al.* 1996) indicating

volume production of 40 m^3 ha^{-1} per year, but there are few estimates of volume produced from large scale plantations at full rotation. Growth can often fall away after the first 3-4 years on shallow, infertile soils. MAI of much over 20 m^3 ha^{-1} on an operational scale over a full rotation would appear to be exceptional, requiring a combination of high-quality sites, best genetic material and good silviculture.

The current best estimates of the climatic range required for good growth of *A. mangium* (adapted from Booth 1996) are as shown below. These estimates have been determined by computer-based analysis of the climates throughout the species' natural range, together with analysis of climatic records from areas where the species is known to perform satisfactorily as an exotic.

Annual rainfall	1500-3000 mm
Length of dry season (consecutive months with less than 40 mm rainfall)	0-6 months
Mean annual temperature	22-30°C
Mean maximum temperature of the hottest month	30-40°C
Mean minimum temperature of the coldest month	9-24°C
Extreme minimum temperature	0°C

A. auriculiformis appears to be able to tolerate a somewhat longer dry season and lower annual rainfall, while *A. aulacocarpa* and *A. crassicarpa* have very similar requirements to *A. mangium*. Enthusiasm for growing tropical acacias is leading to their being planted in much drier conditions, leading to poor yields and complete failure in some cases. Strong wind is another important factor affecting success of plantations and species choice. *A. mangium* with its very heavy canopy and low density, low strength wood is the most susceptible species and is not suitable for planting in cyclone prone areas.

Species characteristics. In general *A. mangium* is usually the fastest-growing species of the four during the first few years, but on sandy, infertile sites, *A. crassicarpa* can grow as fast or faster (Sim 1991). *A. mangium* has the lowest wood density and *A. auriculiformis* the highest as judged from pilodyn measurements (Sim 1991) and during pulping trials (Clark *et al.* 1991, Kha 1996). *A. mangium* tolerates infertile, acid soils, whereas *A. auriculiformis* tolerates alkaline soils (Nikles and Griffin 1992). Although *A. mangium* grows very fast, it frequently forks. Stems of *A. auriculiformis* are very rarely straight whereas *A. aulacocarpa* frequently forms straight, single stems, but does not grow as fast as the other species. *A. crassicarpa* is susceptible to borers (although damage from the pin hole borer is not economically important Sim pers. comm. 1996) and to damage from wind although it does grow fast.

Some of the species readily form hybrids and *A. mangium* x *A. auriculiformis* has demonstrated superiority to the pure species in some circumstances (Pinso and Nasi 1992, Kha 1996).

The hybrid *A. mangium* x *A. auriculiformis* has shown promise for growth in Sabah where it grew 6 & 17% faster for height and 11 & 35% faster for diameter than *A. mangium* on two sites aged 11 & 7 years respectively (Lapongan 1987). In Vietnam (Kha 1996) the hybrid had twice the stem volume of *A. mangium* at 4.5 years and had significantly higher wood density, pulp yield and quality.

Improvement objectives. First, species must be correctly matched to site so that best growth rates can be obtained. Production objectives must be known - pulp, sawn timber, veneer and or fuelwood. In the first 1-2 generations, improvement programs have focussed on increasing stem volume and improving stem form (straight, single stems to improve recovery of solid wood products and reduce harvesting costs for pulpwood). Wood quality (density and pulp yield for pulpwood, and appearance, strength and freedom from defects for solid wood products) will become important objectives in advanced breeding programs.

Provenance results. Provenance trials of *A. mangium* have shown that on most sites the provenances from PNG and south eastern Irian Jaya are the most productive in terms of wood volume (Harwood and Williams 1992, Otsamo *et al.* 1996). Three-year results of an international series of provenance trials of *A. auriculiformis* are less clear-cut (Awang *et al.* 1995). However, in trials in northern Australia, Vietnam, Malaysia and India, the general trend has been for PNG and Queensland provenances to produce the greatest volume, and Queensland provenances to display the best stem form (single, straight main stems). Very poor performance of land races from India and SE Asia has been a feature of some trials (Bulgannawar and Math 1991). Northern Territory provenances have usually displayed slow growth and poor stem form. PNG provenances of *A. crassicarpa* and *A. aulacocarpa* have been found to have superior growth rates than those from Queensland in most provenance trials (Thomson 1994).

An intriguing finding is that these growth patterns for provenance regions of *A. mangium* seems to follow levels of genetic diversity at RFLP loci (Butcher *et al* 1996). Seedlots from the Indonesian island of Ceram and from Sidei in northwestern Irian Jaya had the lowest diversity; provenances from PNG were more diverse than Cape York provenances in turn more diverse than provenances further south in Queensland (Butcher *et al.* 1996).

Seed collection. Seed from the best natural provenances in New Guinea and far north Queensland is expensive to collect and sells at a high price on the international market. Seed production is prolific. Seed orchards of *A. mangium* in northern Queensland have produced annual crops of over 50 kg of seed per hectare from age 5 years on. This makes establishment of seed production areas and seed orchards an early priority in any planting program and improvement strategy.

INTRODUCTIONS

Acacia auriculiformis has been planted as a street tree in southeast Asia for many years and has been well-known as an ornamental in Thailand since 1935 (Pinyopusarerk 1987). The

provenances involved in the initial introductions are not known. Australian acacias were introduced to China in the 1950s, beginning with the temperate species *A. mearnsii*. The tropical species were introduced in the 1960s beginning with *A. auriculiformis* to Guangzhou in 1961 (Pan and Yang 1987). This species is still the most widely planted tropical acacia in the country (50,000 ha plus 3000km roadside plantings) (Wang and Fang 1991). Other tropical species such as *A. mangium* and *A. crassicarpa* were first introduced in 1979.

Acacia mangium was introduced to Sabah (East Malaysia) in 1966 as seed from a single mother tree from the Mission Beach, Queensland provenance, for use in firebreaks (Sim 1987). Successive plantations derived from the original introduction have shown progressive decline in vigour (Sim 1984), most probably the result of buildup of inbreeding depression from this narrow genetic base. Seed from seed orchards derived from this initial introduction performs poorly relative to superior natural provenances such as Oriomo, Papua New Guinea and Claudie River, far north Queensland (Turvey 1995). Seed production areas at Subanjeriji, south Sumatra, were established in 1979 from Julatten, Mossman and Cassowary, inferior provenances in the south of the species range in Queensland. Seed from Subanjeriji has also performed poorly relative to Oriomo and Claudie River (Turvey 1995, Otsamo *et al.* 1996).

Beginning in the late 1980s, large-scale collections of *A. mangium* from many hundreds of trees in have been made in New Guinea and the Claudie River area in Queensland, and these collections have provided much of the seed for recent plantation establishment, providing growers with an excellent broad genetic base from the best provenances for selection. Many organisations have now established their own seed production areas and seedling seed orchards based on these superior provenances, and these stands are starting to yield seed. Similar bulk collections of *A. auriculiformis* and *A. crassicarpa* have also been used for plantation establishment on a smaller scale.

Research organisations and commercial growers in several countries have established genetic base populations in the form of seedling seed orchards, using hundreds of individual-tree seedlots of known natural provenance origin collected by the CSIRO Australian Tree Seed Centre.

WHAT IS IT USED FOR

As a native-forest species, none of the species is much used. In PNG, for example, villagers prefer other species for poles and commercial timber-getters are more interested in other hardwoods. However, most of the new plantations in southeast Asia are to be used for pulp and paper. There are now 15 pulp mills in Indonesia with a capacity of 2m tonnes per year and there are plans to build a further 6 mills by the end of 1999 with a further capacity of 1.9m tonnes. In Sabah, the current SFI mill operates with a capacity of 150k tonnes per year and there are plans for 2 mills in Sarawak with a capacity of 2m tonnes/year. Although these mills do not operate exclusively on plantation acacias, they illustrate the capacity of the region to process plantation-grown wood into pulp. Some of the organisations planting acacias are

planning to produce timber which can be used for solid wood products and veneer, with the residues being used for pulp. In Malaysia and Indonesia, very large expansions of the plantation resource are planned for the year 2010. In Indonesia plantations are planned to be more than three times as large by 2010,whereas in Malaysia, a ten-fold expansion is planned.

Acacia mangium is a light hardwood and has properties comparable with other tropical hardwoods such as *Shorea* species. It has an attractive appearance and makes good furniture, but the prevalence of knots reduces quality (Razali 1993). In Peninsular Malaysia, *A. mangium* was planted in the Compensatory Planting Program for production of timber. Heartrot and soft knots greatly reduced the recovery rates of sawn timber in these plantations (Ho Kam Seng and Sim Heok Choh 1994). *A. auriculiformis* has higher density wood which is suitable for furniture making and other solid wood applications (Pinyopusarerk 1990). The interspecific hybrid between these two species has wood of intermediate density with excellent pulping properties (Kha 1996). Acacias produce fuelwood of acceptable quality, and are used for this purpose in rural areas of many countries including India, Thailand and Vietnam.

BREEDING STRATEGIES

Breeding strategies in outcrossing species are usually a compromise between reducing genetic variability to maximise gain and increasing genetic variability to retain flexibility. Gain is maximised in the short term when the "best" genotype is selected and propagated in a non-varying environment. As environments (both growing and marketing) change, the definition of "best" also changes and so some flexibility is required. Breeding and selection in a population with too little genetic variability frequently leads to inbreeding depression. Hence some level of genetic variability is required to avoid too much inbreeding and to provide for genetic recombination each breeding cycle. Too much genetic variability leads to maladaptation and lack of genetic progress because there is too little genetic selection.

In devising a breeding strategy, we must make use of features of the biology to optimise the gains. These were summarised by Matheson and Sim (1992):

- Prolific in numbers of seeds produced (pioneer species) (more than 100,000 seeds per year per tree)
- Flowers early in life-cycle (seed at about 3 years of age)
- Difficult to control-pollinate
- Difficult to vegetatively propagate on large scale from adult stock
- Can vegetatively propagate on small scale (marcots & cuttings)
- Tissue culture only from embryos
- Can make cuttings of young seedlings
- Hybrid A. mangium x A. auriculiformis seems promising
- Actual status of natural hybrid trees unknown, so it would be unwise to base a breeding program on them.

Matheson and Sim (1992) devised a breeding strategy for the main four tropical acacias to be applied in Sabah which made use of these features as far as was possible. Because controlled pollination is very difficult in these acacias, complete control of pedigree is impractical within a breeding program for these species. Although bulking up small numbers of CP seed through stem cuttings is possible, it takes time; about 1 year to make enough cuttings for 100 ha. The occurrence of malformations following tissue culture (Darus 1992) mean that tissue culture may not be advisable. Early and prolific flowering mean that gain may be more effectively achieved through open-pollination and quick turn over of generations. It is also much simpler and requires much less infrastructure. The strategy published by Matheson and Sim (1992) was therefore based on open-pollination, but made allowance for propagation by stem cuttings of small numbers of control-pollinated seed.

The strategy involved two parallel population combining features of multiple population breeding (Namkoong *et al* 1980) in which populations are subject to slightly different selection and subline breeding in which sublines are separately maintained (without crossing) but within which inbreeding is allowed to increase (Matheson and Brown 1983). The parallel populations could be different lines of the same species for single species breeding or they could be different species where the hybrid shows promise (eg *A. mangium* x *A. auriculiformis*). The scheme is shown in Fig n and begins with 50 plus tree selected for each population. The 10 best of these are marcotted each 10 times to provide material for a clonal seed orchard. The seed orchard is constructed by pairs of trees (one from each population) being planted so close to one another that their crowns intermingle. Because selfing rates are low, most of the seed from these crowns will be from crosses between the two parents. Individuals to make up each pair should be selected so they flower at the same time. From the 50 selections, 30 OP seedlings are selected and 5 cuttings taken from each one to form a clonal progeny trial. These trials are measured and then culled based on the measurements before flowering. The best 50 individuals are then selected to regenerate the population and 30 seeds collected from each, the best 10 trees are marcotted to form clonal seed orchards as before. Selection can be carried out using more than one individual from better families, only one from the poorer families (Lindgren and Matheson 1986). The culled trials can then be used as seed production areas whose seed can be compared with seed from the clonal hybrid orchards. As controlled pollination techniques improve, they can be used to cross populations instead of using the hybrid orchards. New material can be infused at the clonal progeny test stage. Each generation of breeding in this way can be completed in about 5 years with a normal rotation length for pulp and paper of about 8 years.

Another strategy has been to establish seedling seed orchards (SSO) made up of family-identified (usually) seedlots derived from collections from natural provenances. (Harwood *et al.* 1994). These orchards perform the dual purpose of trials for identifying superior provenances and families and are then thinned initially to a single tree in each plot. Further thinning by provenance and family is possible. However, neighbourhood inbreeding in natural stands and differential amounts of selfing among families mean that selection should not be too intense or too stringent. Seedling seed orchards of *A. mangium*, *A. aulacocarpa*, *A. auriculiformis* and *A. crassicarpa* were established in northern Australia over the period 1989-1991 (Harwood *et al.* 1994). A seedling seed orchard of *A. auriculiformis* was

established by the Royal Forest Department, Thailand in 1989, and two second-generation SSOs of this species were planted in Thailand in 1994. More recently, seedling seed orchards and seed production areas of some or all of the four species, using adequate genetic bases of superior provenances, have been established in India, the Philippines, Vietnam and Indonesia, by private companies and government agencies. The FORTIP program, with technical support from ATSC, has collaborated with government research agencies to establish many of these orchards.

Pests & diseases Only a few insect pests are of major importance - root feeders (eg termites), branch and stem borers (*Sinoxylon* spp. and the red coffee borer (Chaweewan Hutacharern 1993).

The most important to date is heart rot, first observed in 1981 in Sabah, then in Peninsular Malaysia. It is most prevalent in trees more than 4 years old. It seems to be associated with branch stubs from self- or artificial pruning (Lee 1993). Although so serious in Peninsular Malaysia that planting has been suspended, it is not thought to be a threat in Sabah. This relates to the rotation length and end use applications: *A. mangium* is grown for timber in Peninsular Malaysia with a rotation length of around 15 years, but for pulp in Sabah with a rotation length about 8 years. *A. auriculiformis* and the hybrid seem to be less susceptible than *A. mangium* and the incidence of the disease seems lower in Sabah than Peninsular Malaysia. Heart rot does not seem to affect the quality of pulp or paper (Sim Boon Liang, pers. comm.)

A range of fungal diseases, including leaf spots, gall rusts, shoot blights, stem cankers, heart rot, and root rots may affect the future productivity of tropical acacia plantations (Old *et al.* 1996). Some fungal diseases may be more prevalent in lowland equatorial south east Asia, where high humidity and rainfall occur throughout the year, than in most of the natural range, where there is a distinct dry season.

CONCLUSION

The four tropical acacias discussed here are very promising species for the humid tropics, particularly Asia and Southeast Asia. They grow very fast, particularly so when young, yet form timber of high density and are suitable for use as pulp. Large areas of plantations are currently being established, and plans are to extend these plantings considerably. Apart from the heart rot which has affected plantations in Peninsular Malaysia, these species have so far remained remarkably free of pests and diseases. Principal traits for improvement are bole length (to reduce forking), stem straightness and branching. The hybrid between *A. mangium* and *A. auriculiformis* shows great promise.

DISTRIBUTION, BIOLOGY, GENETICS, AND IMPROVEMENT PROGRAMS FOR *EUCALYPTUS GLOBULUS* AND *E. NITENS* AROUND THE WORLD

W. N. Tibbits[1], D. B. Boomsma[2] and S. Jarvis[2]

Abstract:- The distribution of natural forests and plantations of *E. globulus* and *E. nitens* is outlined. The total global area of plantations of *E. globulus* was about 1,700,000 ha at the end of 1995, whilst that for *E. nitens* stood at about 150,000 ha. In the period 1991 to 1995, about 350,000 and 100,000 ha of new plantations of *E. globulus* and *E. nitens* respectively were established, largely in Portugal, Spain, Chile, Australia and Uruguay. Based on proposed planting rates, the period 1995 to 1999 may see a further increase of new plantations of about 180,000 and 70,000 ha. The main end-use of plantations is for the pulp and paper industry. When compared on the same sites in Australia, *E. globulus* has on average the higher basic density and slightly higher pulp yield. Biological aspects such as site preferences, growth rates, pests, and diseases, and establishment and management prescriptions are discussed. Information is summarised on genetic control of important traits, and tree improvement programs around the world.

Key Words: *E. globulus*, *E. nitens*, Plantation, Breeding, Wood quality.

DISTRIBUTION

Natural Distribution. *E. globulus* forms a species complex (Jordan *et al.* 1994), with four apparent populations, currently ascribed to four ssp. (ssp.), *E. globulus* Labill ssp. *globulus*, *E. globulus* Labill ssp. *bicostata* (Maiden, Blakely & J. Simm.) Kirkpatr., *E. globulus* Labill ssp. *pseudoglobulus* (Naudin ex. Maiden) Kirkpatr., and *E. globulus* Labill ssp. *maidenii* (F. Muell.) Kirkpatr. These four recognised taxa are largely differentiated on reproductive traits, with *globulus* having umbels of single fruits, *bicostata* and *pseudoglobulus* having three fruits per umbel and *maidenii* having up to seven fruits per umbel. Each taxa has core populations that are geographically separated. However, *globulus* integrades between *bicostata* and *pseudoglobulus*, which themselves dispay a continuum between each other. This paper deals with *E. globulus* ssp. *globulus* (referred to *E. globulus* from here on).

E. globulus, Tasmanian Blue Gum, was one of the first Eucalypts to be formally described and also cultivated as an exotic (Eldridge *et al.* 1992). It occurs along the coast and up to 60 km inland in Tasmania and Victoria, over a latitude range of 38° 30' to 43°30'S and an altitudinal range of sea level to about 600 m (Figure 1).

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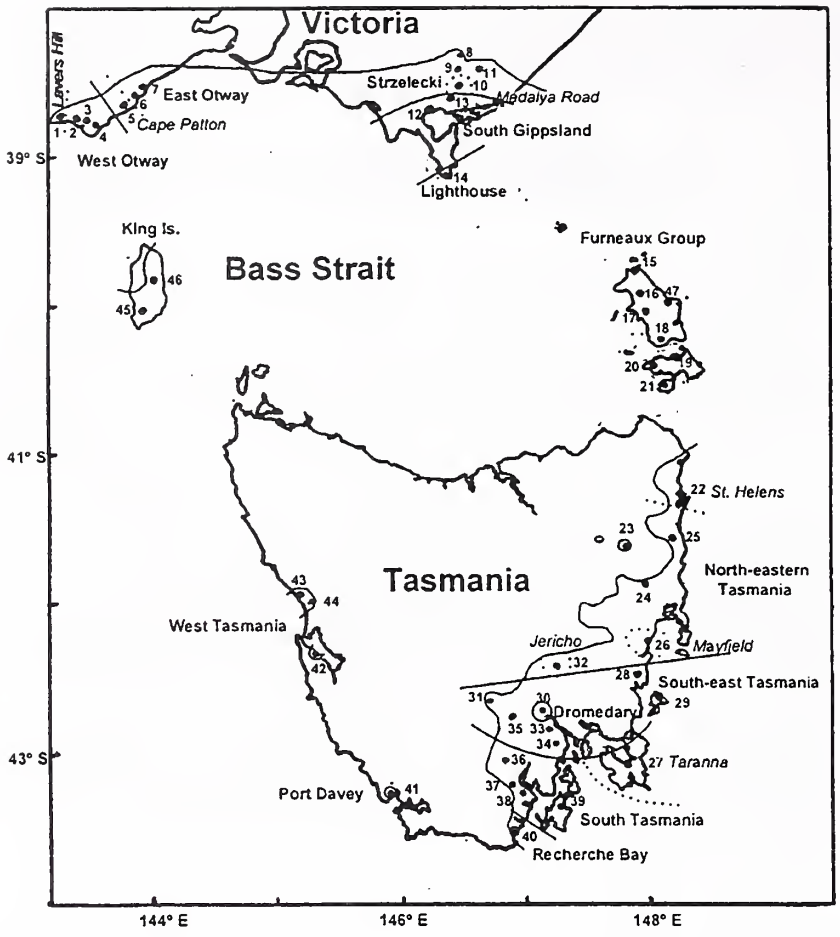

Figure 1. Natural distribution of *E. globulus* (after Dutkowski unpublished).

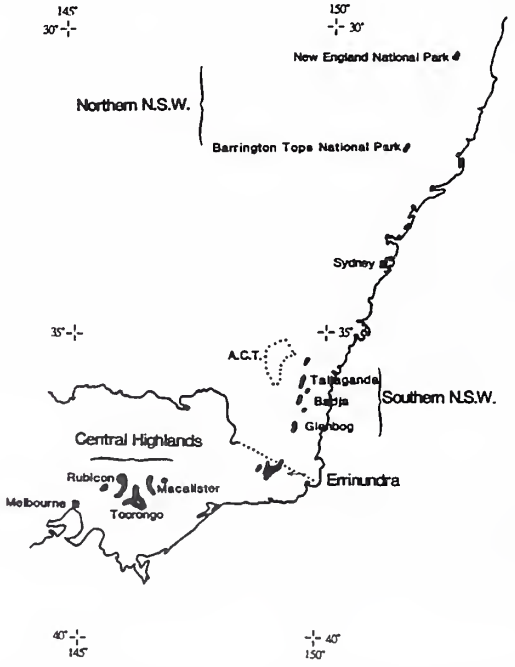

Figure 2. Natural distribution of *E. nitens* (after Tibbits and Reid 1987).

Jordan *et al.* (1994) identified 12 geographic races of *E. globulus*, with three in Victoria [(1) Otway Ranges, (2) Strezlecki Ranges, (3) South Gippsland], two on Bass Strait islands [(4) King Island, (5) Furneaux Group], the (6) central western Tasmania race, four in eastern Tasmania [(7) northeastern Tasmania, (8) eastern Tasmania, (9) Jericho, (10) southeastern Tasmania], and two small races (11) Port Davey and (12) Lighthouse, Wilson's Promontory.

Eucalyptus nitens (Deane & Maiden) Maiden, Shining Gum, is a fast-growing species with a natural distribution in small and generally isolated populations. It occurs in the mountain ranges of New South Wales (NSW) and Victoria, over a latitude range of 30° to 38°S and an altitudinal range of 600 to 1500 m (Figure 2). Pederick (1979) determined four main regions of occurrence, two in NSW (northern NSW and southern NSW) and two in Victoria (east Gippsland and the central highlands). Within the central highlands he designated three provenances, Rubicon, Toorongo and Macalister, and he designated the other regions as provenances. Pederick (1979) described two forms of *E. nitens*. The "juvenile-persistent" form was so called because of its retention of juvenile foliage after the first year of growth. The "early-adult" form did not retain its juvenile foliage for long. It came from the Errinundra provenance and parts of the Toorongo provenance, and was characterised by slower growth, finer branching and straighter stems (Pederick 1979). It has been found to have poorer cold hardiness (Tibbits and Reid 1987), different floral morphology (Tibbits 1989) and it appears to be characterised by lower pulp yields (Williams *et al.* 1995). It has subsequently been determined to be genetically distinct, and is classified by some taxonomists (Cook and Ladiges 1991) as a different species *E. denticulata*.

Area Planted. The area planted to *E. globulus* and *E. nitens* around the world, has been estimated from published information and responses from a questionnaire sent to organisations. Of the two species, *E. globulus* has by far the largest current total area with about 1,700,000 ha at the end of 1995 (Figure 3a). This is about an order of magnitude bigger than that for *E. nitens*, which stood at about 150,000 ha. Hence, in terms of area planted, *E. globulus* is more significant than *E. nitens*. This is apparently a consequence of a general preference for organisations to establish *E. globulus*, due to its favoured wood properties (see below), and also due to its earlier introduction as a plantation species. *E. nitens* is often planted on sites unsuitable for *E. globulus*.

Plantation estimates for *E. globulus* in some countries like Ethiopia, Peru, Colombia and Ecuador are uncertain, and the cumulative figures for these countries reported here account for 30% of the total area (Neilson and Manners 1997). However, where more reliable estimates exist, the most significant areas are undoubtedly in Spain and Portugal, each of which has about equal areas, which together account for 60% of the total world planting of *E. globulus*. The next largest planting is in Chile. China, Australia and Uruguay have approximately equal areas. A number of organisations in Spain and Portugal (Iberia) have plantation estates of *E.* globulus at about 50,000 to 100,000 ha. However, it would appear that the majority of area in Iberia is in very small plantings of a few hectares owned by farmers. In the period 1991 to 1995, about 350,000 ha (20% increase) of new plantations were established, largely in Iberia, Chile, Australia and Uruguay. Based on information supplied by organisations on

proposed planting rates, the period 1995 to 1999 may see a further increase of new plantations of about 180,000 ha (10%) to a total of 1,900,000 ha.

E. nitens is concentrated in fewer countries than *E. globulus* (Figure 3b). These are countries where reliable estimates generally exist. The most significant plantings are in Chile with 67,000 ha (45%), Australia with 46,000 ha (30%) and South Africa 26,000 ha (17%). Unlike *E. globulus,* very few of the *E. nitens* plantings are of a few hectares owned by farmers, but are larger industrial plantings. However, only a few organisations have plantation estates of *E. nitens* which exceed 15,000 ha. In the period 1991 to 1995, about 100,000 ha (160% increase) of new plantations were established, largely in these three countries. Based on information supplied by organisations on proposed planting rates, the period 1995 to 1999 may see a further increase of new plantations of about 70,000 ha (45%) to a total of 220,000 ha.

End-use Focus. The predominant end-use of cultivated *E. globulus* is for the pulp and paper industry. For instance, in Portugal with about 550,000 ha of *E. globulus*, the average use by the pulp and paper industry in 1995 and 1996 was about 3,500,000 m^3 of wood free of bark, which was used to produce about 1,100,000 tonnes of kraft pulp (the majority being bleached pulp). In Spain, with a similarly large area planted, about 3,300,000 m^3 of wood is currently harvested for the pulp and paper industry (Neilson and Manners 1997). In Chile, the figure is about 1,500,000 m^3 (mostly *E. globulus*) and is forecast to rise (*E. globulus* and *E. nitens*) to something similar to that of Portugal or Spain by the year 2000 (Neilson and Manners 1997). Of the industrial growers of *E. globulus* surveyed, only in China, Argentina and Australia were other end uses indicated. The plantings in China are in Yunnan Province, some distance from any potential pulp and paper processing, and the end uses are posts, sawn timber and fuel wood. In Argentina, it is estimated that 10% of the wood is destined for posts, sawn timber and fuel. Two of 13 Australian organisations indicated that about 5 and 80% of the wood yet to be harvested would possibly end up as sawn timber. The mix of end uses of *E. nitens* is a slightly different story, since only about 4,500 ha is currently harvested annually. The majority of this is in South Africa, where 25% of the wood is used as posts and 75% for the pulp and paper industry. A similar mix of end uses in China exists as for *E. globulus*. Two of seven Australian organisations with *E. nitens* indicated that about 20 and 80% of the wood yet to be harvested would possibly end up as sawn timber, and one indicated about 15% use for veneer.

BIOLOGY

Site Parameters. Eucalypts are fairly site sensitive requiring appropriate matching of species to the climatic factors of the sites. Both *E. globulus* and *E. nitens* are species that perform well in cool temperate climates. The broad latitudinal ranges for *E. globulus* and *E. nitens* are about 30 to 40°N or S, and 35 to 45°N or S, respectively. The species are planted at more equatorial latitudes in places like China, Colombia, Ecuador and South Africa, but at much higher elevations with a temperate climate. The mean annual temperature at these sites is of the order of 11 to 16°C for both species, although in Tasmania, Australia, *E. nitens* is on some sites with a mean annual temperature as low as 8°C (Table 1).

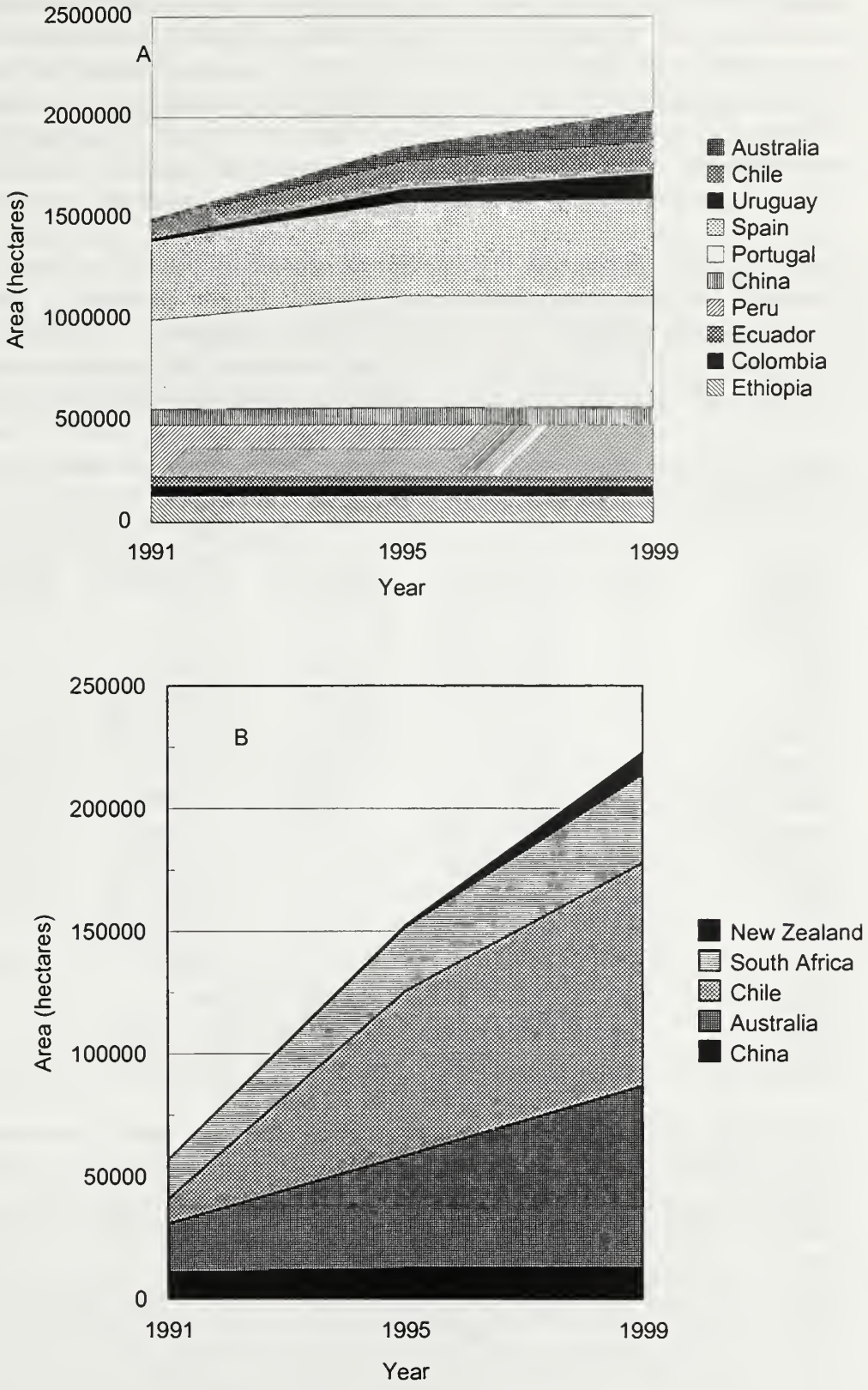

Figure 3. Areas of plantations by country and year for (A) *E. globulus* and (B) *E. nitens*.

E. globulus is generally planted on sites where mean annual rainfall ranges from a low of 500 mm to a high of 2000 mm (Table 1). Whilst *E. nitens* is planted on sites with similar rainfall parameters (Table 1), it tends to be on wetter sites. This would appear to be related to *E. nitens* being established on some higher elevation sites, due to its greater cold hardiness, and the correlated response of increased rainfall with elevation. The establishment of *E. globulus* on the drier sites would appear to support the findings of Honeysett *et al.* (1996) that *E. globulus* is more suited than *E. nitens* where moderate levels of water stress are experienced, and may use less water per unit of volume growth in the early years than *E. nitens*. In the same geographic area, *E. globulus* is generally planted on lower elevation sites than *E. nitens* (Table 1). For instance, in Chile *E. globulus* is generally planted on elevations up to 450 m, whilst *E. nitens* is established up to 800 m elevation. In Tasmania, Australia, *E. globulus* is generally planted on elevations up to 300 m, whilst *E. nitens* is established up to 700 m elevation range. This is related to the greater relative cold hardiness of *E. nitens*. In winter seedlings of *E. nitens* may be about 4°C more hardy (Tibbits *et al.* 1991).

Table 1. Ranges in climatic parameters for areas of some countries where *E. globulus* (g) and *E. nitens* (n) are planted. NZ = New Zealand.

		Parameter			
Country	Species	Rain (mm)	Latitude (°)	Altitude(m)	MAT (°C)
Argentina	g	900 - 1000	37 ½ - 39 S	0 - 150	13 - 14
Australia	g	600 - 1600	32 - 43 S	0 - 700	11 - 15
Chile	g	500 - 2000	33 - 41 S	0 - 450	11 - 15
China	g	800 - 1000	24 - 30 N	1500 - 2000	14
Colombia	g	1500 +	2 ½ N	2400 - 2800	10 - 12
Portugal	g	600 +	37 - 42 N	0 - 400	12 - 17
Spain	g	700 - 1800	37 - 44 N	0 - 1000	12 - 17
Uruguay	g	1000 - 1200	30 - 34 S	100 - 200	15 - 16
Australia	n	650 - 2000	36 ½ - 43 S	0 - 1100	8 - 15
Chile	n	800 - 2000	35 - 42 S	0 - 800	11 - 15
China	n	800 - 1100	24 - 30 N	1500 - 2000	14
NZ	n	1400 - 2100	37 ½ - 38 S	0 - 600	14 - 16
Portugal	n	600 +	unclear	400 +	12
Spain	n	900 - 2000	43 - 44 N	400 - 1000	12
South Africa	n	800 - 1100	26 - 35 S	1300 - 1600	12 - 16

Growth Rates. Figure 4 presents the range and average mean annual increment (MAI) for both species, on the basis of some published information and that supplied by organisations planting the species. The more reliable estimates of MAI for *E. globulus* appear from Portugal and Spain, where large areas and volumes are harvested. In Portugal the MAI ranges from 5 to 12 and averages 9 m^3 ha^{-1} $year^{-1}$ (Neilson and Manners 1997). Information from Portugal is largely based on under bark volumes for coppice rotations of about 10 years. Genetically improved trees scheduled to be harvested near the end of the century, are estimated by organisations such as Stora Celbi to average 16 m^3 ha^{-1} $year^{-1}$ compared with 10 m^3 ha^{-1} $year^{-1}$ for genetically unimproved trees. In neighbouring Spain, MAI for *E. globulus* is reported to range from 10 to 23 and average 16 m^3 ha^{-1} $year^{-1}$ (Neilson and Manners 1997). Information for other countries appears to be somewhat varied in availability and reliability. In

Chile the *E. globulus* currently being harvested is reported to have a lower than expected range in MAI of 12 to 18 m^3 ha^{-1} $year^{-1}$ due to poor initial management, low survival, effects of cold, and poor soils (Neilson and Manners 1997). However, five major organisations in Chile supplied information to the authors suggesting anticipated MAI averaging about 30 m^3 ha^{-1} $year^{-1}$. Similar estimates were supplied from organisations in Argentina and Uruguay. In Australia, MAI is based on sparse evidence, but all ten organisations listed their average MAI as 20 to 28 m^3 ha^{-1} $year^{-1}$. Estimated MAI for China is quite low at about 8 m^3 ha^{-1} $year^{-1}$, which may be due to relatively poor soils.

There are few reliable estimates of MAI for *E. nitens* since there is little at rotation age or being harvested. However, in South Africa both organisations Sappi and Mondi report that MAI is about 13 to 14 m^3 ha^{-1} $year^{-1}$. In Tasmania, Australia, the organisation with the largest global plantation estate (30,000 ha), North Forest Products, lists its MAI at 18 m^3 ha^{-1} $year^{-1}$, on the basis of extensive inventory plots and a recent commencement of harvesting of small areas. In Chile, the anticipated MAI is generally equal to *E. globulus* (30 m^3 ha^{-1} $year^{-1}$). Similar estimates were supplied from New Zealand (25 m^3 ha^{-1} $year^{-1}$). In Australia, the average MAI for nine organisations was 20 m^3 ha^{-1} $year^{-1}$. As for *E. globulus,* MAI for China is quite low at about 9 m^3 ha^{-1} $year^{-1}$.

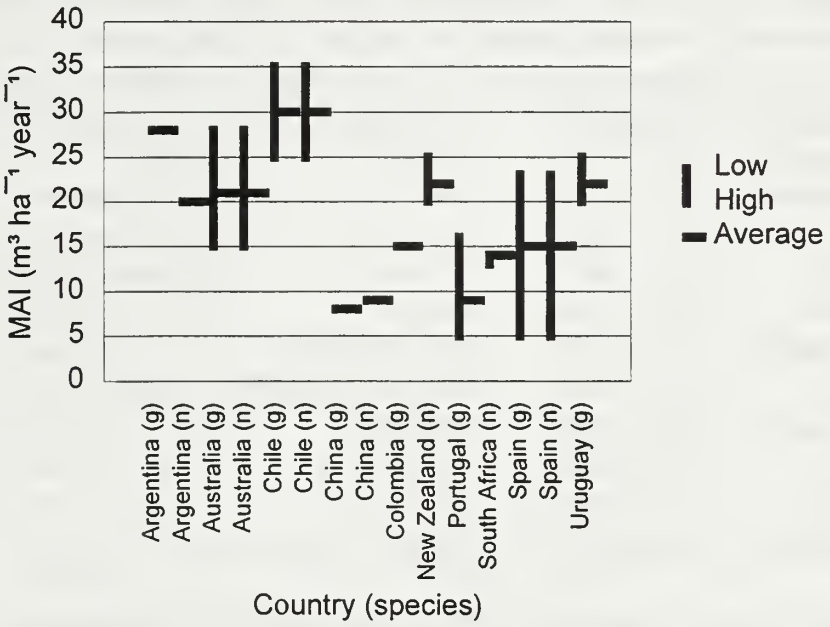

Figure 4. Mean annual increments (MAI) for *E. globulus* (g) and *E. nitens* (n).

Establishment and Management Prescriptions. Cultivation, stocking, fertiliser application, weed control, rotation length, harvesting prescriptions and fire control measures are generally much the same for either *E. globulus* or *E. nitens* for a given organisation. There are various differences amongst organisations and countries. Some are summarised in Table 2. Weed control (not shown in Table 2) is generally with a combination of chemical and mechanical measures often aimed at 100%

control for the first year or two. The effectiveness of chemical weed control can be varied (Wilkinson and Neilsen 1990). No weed control is reported used in China.

Pests and Diseases. A total of 17 types of pests and diseases were listed by organisations planting *E. globulus* and *E. nitens* (Table 2). The greatest number are reported in Australia, where the species naturally occur. Mortality due to browsing by mammals may cost about US $ 100 ha^{-1} in lost net present value (Montague 1996).

Reproductive Biology and Vegetative Propagation. Trees of both species may flower early, often as young as about four years of age. However, quantity and consistency of flower crops have sometimes been a problem. Studies have shown that certain chemicals can be applied to promote flowering (Reid *et al.* 1995). Successful controlled pollination techniques exist for both species (Hardner 1996; Tibbits 1989). Generally *E. nitens* does not propagate readily using cuttings (Tibbits *et al.* 1997) or micropropagation. *E. globulus* propagates more easily, particularly with cuttings.

Table 2. Establishment and management prescriptions for organisations in some countries where *E. globulus* and *E. nitens* are planted. Pests : 1 (Autumn Gum Moth, *Mnesampela privata*), 2 (Leaf Beetles), 3 (Sawfly, *Perga* spp.), 4 (*Gonipterus scutellatus*), 5 (*Phoracantha semipunctata*), 6 (ants or other insects), 7 (Parrots), 8 (marsupials and other mammals); diseases : 9 (*Mycosphaerella* spp.), 10 (decay fungi), 11 (*Chilecomadia valdiviana*), 12 (*Cryphonectria cubensis*), 13 (*Septoria pulcherima*), 14 (*Botrysphaeria dothidea*), 15 (*Aulographina* spp.), 16 (*Endothia gyrosa*), 17 (Gum tree scale). NZ = New Zealand, SA = South Africa.

Country	Trees ha^{-1}	Fertilizer (g $tree^{-1}$)	Rotation (y)	Pests /Diseases
Argentina	1,333	unclear	10	none reported
Australia	800 - 1299	100-200	8-25 pulp 40 timber	1,2,3,4,5,6,7,8, 9,10,15,16,17
Chile	1111 - 1667	180-300	10-15	2,5,9,11
China	2200 - 2500	manure	15	none reported
Colombia	1111	100 + 100 NPK	7	9
NZ	1,200	60-200 urea,DAP	10-12	2,8,9,13
Portugal	1000 - 1200	250 NPK	12	5
SA	1143 or 1667	50-100 DAP	10	4,9,12,14
Spain	640 1429 1667	100 NPK	12-18	4,5
Uruguay	unclear	unclear	10	6

Wood Quality Comparisons. There is little published information on the wood quality parameters for pulping of cultivated *E. globulus* and *E. nitens*. Neilson and Manners (1997) list basic densities and kraft pulp yields for harvested *E. globulus* of generally unspecified ages in Spain, Ecuador and Chile ranging from about 560 to almost 600 kg m^{-3} and 54 to 58% respectively. The New Zealand Forest Research Institute (Anon. 1996) list a range in basic densities and kraft pulp yields for twenty 15-year-old *E. nitens* of 435 to 542 kg m^{-3} and 54 to 59% respectively. Basic density and cellulose (from NIRA methodology) at two sites in South Africa for *E. nitens* trees almost at rotation age averaged 540 kg m^{-3} and 52% respectively (ICFR 1993). Site effects can also result in highly significant variation in wood quality. Williams *et al.* (1995)

reported maximum differences in kraft pulp yield of 2.4% points for *E. globulus* and 2.5% points for *E. nitens* over three and four sites.

Direct comparisons of the two species growing well on the same site have seldom been reported. A study by Williams *et al.* (1995) found that at three Tasmanian sites from 60 to 440 m altitude, at eight years of age, kraft pulp yield was generally higher, whilst basic density and pulp productivity (basic density multiplied by pulp yield) were always higher, for *E. globulus* than *E. nitens* (average 54.2% *c.f.* 52%, 503 *c.f.* 459 kg m^{-3} and 272 kg m^{-3} *c.f.* 239 kg m^{-3} respectively). Another study at six years of age on a single Tasmanian site found that whilst kraft pulp yields were approximately equal, basic density and pulp productivity were always higher for *E. globulus* (Tibbits *et al.* 1995). Unpublished data of North Forest Products from seven other Tasmanian sites with trees ranging in age from 5 to 17-year-old supports the generalization that *E. globulus* has about 10% higher basic density and pulp productivity (based on volume, m^{-3}), but only slightly higher kraft pulp yield. There is little information on other pulping processes and relative fibre properties of importance for paper-making. Williams *et al.* (1995) found that cold soda (not kraft) pulps of *E. globulus* had inferior unbleached brightness and light scattering properties, and similar or slightly higher relative energy requirements than *E. nitens*

Table 3. Heritability estimates for growth and wood traits in *E. globulus* (g) and *E. nitens* (n). *r* is the coefficient of relationship assumed for open-pollinated progeny, whilst CP is for controlled-pollinated progeny. dbh = diameter at breast height, BA = basal area, vol = volume, pilo = pilodyn, BD = basic density and PY = pulp yield.

Species	Reference	*r*	Trait	Age (y)	h^2
g	Volker *et al.* 1990	0.4	vol	6	0.19
g	Borralho *et al.* 1992b	0.33	BA	3 - 8	0.15 - 0.19
g	Ipinza *et al.* 1994	0.35	dbh	4	0.13
g	Hodge *et al.* 1996	0.5	vol	2	0.10 - 0.26
g	Hodge *et al.* 1996	CP	vol	2	0.13
g	MacDonald *et al.* 1997	0.4	dbh	4	0.2
n	Whiteman *et al.* 1992	0.4	dbh	9	0.18
n	Hodge *et al.* 1996	0.5	vol	2	0.25
n	Hodge *et al.* 1996	CP	vol	2	0.15
n	Johnson 1996	0.4	dbh	5	0.14
n	Tibbits and Hodge unpubl.	0.5	BA	4 - 7	0.19
g	Dean *et al.* 1990	0.4	PY	8	0.57
g	Dean *et al.* 1990	0.4	BD	8	0.78
g	Borralho *et al.* 1992a	0.33	BD	8 - 9	0.65
g	MacDonald *et al.* 1997	0.4	pilo	6	0.33
n	Greaves *et al.* 1995	0.4	pilo	7	0.6
n	Tibbits and Hodge unpubl.	0.5	PY	6 - 8	0.37
n	Tibbits and Hodge unpubl.	0.5	BD	6 - 8	0.42

Hybrids. There has been considerable interest in assessment of hybrids, since they can be readily produced (Tibbits 1989). Generally speaking F_1 hybrids display intermediate characteristics to both parents (Tibbits *et al.* 1991). The hybrid between these two species is being investigated in Australia, Chile and Portugal. The only

operational planing apparently taking place in by Mondi in South Africa (< 5,000 ha), using the combination of *E. nitens* and *E. grandis*. This hybrid is being tested in New Zealand. The wider use of hybrids appears limited to problems with vegetative propagation (Tibbits *et al.* 1997).

GENETICS

Genetic Parameters. In recent years various estimates of heritabilities and genetic correlations for growth, wood quality and other traits have been published. For growth traits, the heritability estimates average about 0.1 to 0.2 for both species (Table 3). However, for wood properties such as basic density, including its indirect assessment using pilodyn, and pulp yield, heritability estimates range from 0.33 to 0.65. It is beyond the scope of this paper to discuss estimates of genetic correlations. Apparently parameter estimates using progeny from native forests may be affected by varied levels of inbreeding and outcrossing. Borralho and Potts (1996) have suggested that in *E. globulus* the growth of native forest open-pollinated families was affected by the density of stand from which the seed originated and corresponding potential differences in levels of inbreeding. Hardner (1996) tested this hypothesis using isozyme markers, and found that lower breeding values were generally associated with lower rates of outcrossing. No similar work has apparently been undertaken or published for *E.* nitens. Selfing is reported to affect seed set (Hardner and Potts 1995; Tibbits 1989) and early growth (Hardner and Potts 1995; Hardner 1996) in both species. Molecular markers have been developed for *E.* globulus (Vaillancourt *et* al. 1995) and *E. nitens* (Byrne *et al.* 1994), but appear to not be in operational use.

IMPROVEMENT PROGRAMS

Commencement of Breeding Programs. Basic statistics related to breeding programs were received from Chile (6), Australia (4), Argentina (1), Portugal (2), Spain (2), New Zealand (1) and South Africa (2). In many instances the information provided in response to questionnaires was incomplete, but nevertheless it was possible to draw a general picture on current activities. The earliest programs in *E. globulus* commenced in the late 1960's (one in Portugal and one in Australia), with the majority beginning in all countries in the late 1980's and early 1990's. For *E. nitens* the earliest program began in the mid 1970's in Australia, but the bulk of programs in all countries commenced in the early 1990's. Breeding cooperatives for these species began in Chile, Australia and New Zealand around 1990.

Breeding Program Size and Generation Information on the size of breeding populations was limited to the number of families or local selections initially under test. For *E. globulus* the smallest initial population size was 145 families, with the majority of programs having 200 to 800 families under test, and four programs had more than 800 families. For *E. nitens*, the initial populations were generally smaller than for *E. globulus*, with the majority of programs having 200 to 500 families under test and only one program with near 800 families. For *E. globulus*, five of the programs (33%) are at the first generation, eight (*c.* 050%) are at the second generation and two (*c.* 15%) are moving to the third generation. For *E. nitens* seven of the programs (*c.* 50%) are at the first generation, five (*c.* 33%) are at a second

generation and two (*c.* 25%) are into a third generation. Generation interval for both species varied from 4 to 20 years with many in the 8 to 12 years range. Nine out of the 15 *E. globulus* and eight out of the 14 *E.nitens* breeding programs, or just over half the programs, are planning to use controlled pollination (CP) mating schemes.

Table 4. Production Populations (% annual area planted) used currently, and estimate for five years time (shown [0-100] if any change). Sources are ; Prov. = selected native provenance, Race = land race, SPA = seed production area, SSO = seedling seed orchard, CSO = clonal seed orchard, CP = control pollinated seed, clone = stem cuttings. ID is organisation shown in acknowledgments. NZ =New Zealand.

		Source						
Country	ID	Prov.	Race	SPA	SSO	CSO	CP	Clone
E. globulus								
Chile	C1			20[0]		80		[20]
	C2		100[0]			[100]		
	C3			100				
	C4	38[0]	50[0]		12[10]	[70]		[20]
Australia	A1	95[0]		[30]	[30]	[40]		
	A2	40[0]		30	30[50]	[50]		
	A3	74[0]			25[10]	1[85]		[5]
	A4	100[50]			[50]			
	A5				100[75]	[20]	[5]	
	A6	100[40]		[60]				
Portugal	P1						100	
	P2					50[20]		50[80]
Spain	S1	50[15]	20[0	[7]				30[78]
	S2	60[0]	40[0]	[50]	[50]			
E. nitens								
Chile	C1	30[0]		70[100]				
	C2	100				[100]		
	C3			100				
	C4	100[0]			[80]	[20]		
Australia	A2				100[50]	[50]		
	A5	40[0]			100[50]	[50]		
	A7				100			
NZ	NZ1				100[30]	[70]		
Portugal	P1				100			
Spain	S1	100		[50]	[50]			

Breeding Objectives, Traits, Selection Criteria and Analytical Methods. For both species, pulpwood production was the primary focus and was expressed in a number of ways by different companies. The companies in Chile had all included frost tolerance in the breeding objectives for *E. globulus* and *E. nitens*. Sappi from South Africa mentioned fibre quality in their breeding objective for *E. nitens*. Growth was assessed by all programs and measured as volume, basal area, diameter or height for *E. globulus* and *E. nitens*. Half the programs also measured wood density in both species, although it was not clear how many used direct or indirect (pilodyn) approaches. Pulp yield was reported considered or assessed in about 33% of the

E. globulus and 50% of the *E. nitens* programs. Stem straightness and disease resistance were assessed for both species in three programs and one program respectively. Frost resistance in *E.globulus* was assessed by three companies in Chile and one in Australia; for *E. nitens* five companies measured frost resistance. If parent trees were being selected in landrace stands then the age of selection was generally greater than 10 years for half the *E.globulus* breeders, and as old as 40 years for one company. Age of selection for *E. nitens* was generally younger than for *E.globulus* reflecting the younger age of *E. nitens* estates. Age of selection from progeny trials was between 4 and 10 years for both species with selection for frost resistance at less than one year for one company. All breeding efforts are using Best Linear Prediction (BLP), Best Linear Unbiased Prediction (BLUP) or Selection Indices (SI) to rank individuals. Parameters are estimated using Restricted Maximum Likelihood (REML), Gibbs Sampling or SAS procedures, including GLM and VARCOMP.

Production Populations. Table 4 shows the current (1997) and predicted (2002) sources of propagation for some companies growing *E. globulus* and *E. nitens*. Currently for *E. globulus*, there are quite clear differences between countries in the origin of seed and propagules for plantations. In Chile, landrace seed is still important followed by seed from seed production areas. In Spain, selected provenance and landrace origin seed is complemented by some clonal propagation. In Portugal, clonal seed orchards, clonal forestry and mass production of CP seed make up the sources of propagules. In Australia, selected provenance seed (from the native forest) is dominant, followed by seedling seed orchards. Currently for *E. nitens*, in Chile and Spain, again as for *E.globulus*, native provenances and seed production areas supply the seed. Australia, New Zealand and Portugal rely on seedling and clonal seed orchards. In Australia, selected provenance seed (from the native forest) is dominant, followed by seedling seed orchards.

In five years time, all organisations anticipate a shift upwards in genetic quality of propagules. For *E. globulus*, one company in Spain will rely on seedling seed orchards and seed production areas, while the other will move to clonal propagation. Portuguese companies will use clonal propagation and mass CP. Companies in Chile predict that most of their seed will come from clonal seed orchards supported by seedling seed orchards and clonal propagation. In Australia, seedling seed orchards are expected to dominate complemented by clonal seed orchards. For *Eucalyptus nitens*, there is an expectation that seed will be the source of propagules in contrast to *E.globulus*. Seedling seed orchards are expected to dominate in most countries, complemented by seed production areas.

Research Efforts and Priorities . Research priorities listed by 25 organisations (industrial companies, cooperatives and research institutions) from seven countries are listed in Table 5. This should be regarded as a broad picture, since this does not cover all major organisations, and ten of the 25 are from Australia. Nonetheless, the research area of greatest interest appears to be that of wood quality, since it was listed as a priorities in all seven countries and by 15 of the 25 organisations. There are a number of other research areas with apparently similar priority, such as cloning technology, nutrition, pests and diseases, reproductive biology, and weeds. management.

Table 5. Research priorities for organisations (Org.) in Argentina (Ar), Australia (Au), China (Ch), New Zealand (NZ), Portugal (Pt), South Africa (SA) and Spain (Sp).

Research Area	Specific Issues	Org.	Countries
Wood quality	density, pulp yield, fibre properties, mechanical pulping, sawn timber	15	Ar, Au, Ch, NZ, Pt SA, Sp
Cloning	cuttings, micropropagation, embryogenesis	8	Au, Ch, Pt
Nutrition	nutrient balance, fertilizer	6	Au, Ch, Pt
Pests and diseases	management,seed predation, decay	6	Au
Reproductive biology	floral induction, outcrossing rates, orchard management, synchrony, genetic parameters	6	Au, Ch
Weed management	control, prescriptions, cost effectiveness	6	Au, Ch
Silviculture	second rotation, spacing, coppice	5	Au, Ch
Molecular biology	marker-aided selection, genetic engineering	4	Au, Pt
Water relations	drought tolerance, hydrological balance, salinity	4	Au, Pt
Hybrids	testing,clone adaptation	3	Ch, NZ, SA
Genetic parameters	statistical tools, selection, crossing, testing, inbreeding	2	Au

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A REVIEW OF THE WORLDWIDE ACTIVITIES IN TREE IMPROVEMENT FOR *Eucalyptus grandis, Eucalyptus urophylla* AND THE HYBRID UROGRANDIS

Dr. J.A. Wright

Abstract:--The most commonly utilized hardwood plantation species for pulp and paper production are *Eucalyptus grandis, Eucalyptus urophylla* and their hybrid, urograndis. These species have been developed in a large number of countries due to their rapid growth in areas that receive more than 700 mm of rainfall and that have little or no frost. In addition, the species produce seed in a number of locations when planted as exotics and can be cloned using rooted cuttings. Their adequate wood properties mainly for pulp and paper but also for lumber have made them highly desirable and relatively well researched compared to the many hundreds of other eucalypt species. This paper is a review of the worldwide activities in tree improvement with *Eucalyptus grandis, Eucalyptus urophylla* and their hybrid, urograndis.

Keywords: tree improvement strategy, cloning, seed production, plantation development, gene conservation

INTRODUCTION

The native range of *Eucalyptus grandis* includes the states of Queensland and New South Wales in Australia with a range of south latitude from 18 to 33 degrees, a range of altitude from sea level to 1100 m, and a range of annual precipitation from 690 to 2480 (Eldridge et al., 1993). In the native range *E. grandis* receives very few frosts and this affects the areas where the species can be planted.

For *E. urophylla* the native range includes a number of islands in Indonesia of which the most important are Wetar, Timor, Flores, Adonara, Pantar, Alor and Lomblen. The altitudinal range of the species is the largest of any eucalypt species and is from 70 to 2690 meters. Annual rainfall varies from 600 to 2500 mm while the range of south latitude varies from 7.5 to 10 degrees (Eldridge et al., 1993).

FAO (1993) gives 10.06 million hectares of eucalypt plantations outside Australia as of 1990. The species *E. grandis* and *E. urophylla* are amongst the most frequently planted eucalypts for industrial processing in the pulp and paper industry though recent large scale planting in China may alter this. The hybrid urograndis occurs in fewer countries given the cost of making control cross seed, the need to have both of the pure species to make the cross and the lack of control cross technology. The author could only find reports of urograndis plantations in the following countries: South Africa, Brazil, Congo, China, Mexico, Colombia and Venezuela.

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BRIEF HISTORY OF UTILIZATION OF THE SPECIES

Both *E. grandis* and *E. urophylla* have had taxonomic histories that led to confusion in early years. Much of what was called saligna in South Africa and Zimbabwe was *E. grandis* and confusion still exists over saligna seed from southern Africa appearing on the seed label when the trees are actually *E. grandis*. In the case of *E. urophylla* there is still an effort to separate this taxa into different species. In addition the seed originally imported into Brazil was collected from two trees in the Bogor Botanical Garden with the label *E. alba* though the trees were probably pure *E. urophylla*.

Commercial planting with *E. grandis* began in the late 19th century in South Africa (Poynton, 1979) with the intention to be used as mining timber due to shortages of wood from native forests. Plantations were later developed in Europe, the America's, Asia and the less arid areas of Africa. Exact records of introductions are usually not available but much of the seed was obtained from Coff's Harbour, Queensland, Australia.

The commercial planting with *E. urophylla* from collections made in Indonesia began less than thirty years ago though the Dutch colonizers in Indonesia did use the species for ornamental purposes in Jakarta and the Bogor Botanic Garden both on the island of Java where the species is not indigenous. Seed was collected from the trees in the Bogor Botanic Garden and planted in Rio Claro, Brazil. Following adequate flowering and seed production, seed from arboretum trees at Rio Claro, Brazil were used for plantation establishment for many years (Eldridge et al., 1993). This seed would have been hybrid in many cases since many of the eucalypts in the Rio Claro arboretum flower during the entire year. More recently large commercial seed collections have taken place on the islands of Flores and Timor.

Pioneering work in Brazil in the early part of the 20th century by Edmundo Navarro de Andrade led to the publication in 1939 of his book *O Eucalipto*. The first eucalypt plantations in Brazil were for fuelwood and were established by railroad companies. These plantations were from a genetic base of two trees in the Rio Claro arboretum pollinated by surrounding eucalypts. Seed was then collected from selected trees in these plantations for establishing new plantations. After several cycles there was a decline in productivity with almost 15% of the trees having stunted development and the cause was related matings along with inbreeding depression. Given the tax incentive program for plantation establishment which began in Brazil in the 1960's and the poor quality of local seed, large eucalypt seed importations were undertaken from Zimbabwe and South Africa. Seed was also imported from the natural range in Australia but this seed was not available in large quantities and produced trees with considerable disease problems. In the 1970's Champion Papel e Cellulose began mother tree collections of *E. grandis* in Australia and Aracruz undertook collections of *E. urophylla* in Indonesia. At the same time there were collections being made by CTFT of *E. urophylla* in Indonesia for trial establishment in the Congo.

Both species have been included in species trials in many countries. Provenance and progeny trials have been undertaken on a large scale in a substantially smaller number of countries. It is presumed that only those countries where the species have been planted on a semi-commercial scale can the expense be justified to acquire seed from the native range for trial establishment. One of the reasons why *E. urophylla* has not been planted more widely is the continuing difficulty to obtain seed both

from the native range and/or from existing tree improvement programs.

Two of the reasons that *E. grandis* and *E. urophylla* have been used commercially are their propensity to produce seed in a diversity of climate and soil conditions worldwide as well as the relative ease with which these species can be propagated in forest seedling nurseries. It is no accident that the tree species commercially planted all produce seed and can be produced in non-capital intensive nursery conditions.

TREE IMPROVEMENT STRATEGIES

It appears from the literature (Poynton, 1979) that the first formal tree improvement activity with *E. grandis* began in South Africa in the late 1950's while activity in tree improvement with *E. urophylla* began in the late 1960's in the Congo (Eldridge et al., 1993) and in the 1970's in Brazil. The first report in the literature for urograndis was from Brazil. For both species it can be generally stated that initial tree improvement efforts were undertaken by government agencies whereas today private industry accounts for the majority of activity.

Differences in eucalypt tree improvement strategies result from the need to produce wood of short fiber species, existing genetic base, experience of staff and funding. Presently, organizations are involved with tree improvement with these species from those in the stage of initial seed introductions to others with biotechnology including operational use of tissue culture and trials with transformed plants. These strategies can be placed in five categories:

1. Initial Introductions
2. Improved Seed
3. Cloning and Beyond
4. Tree Improvement
5. Species and Provenance Diversification

Initial Introductions. To match climate and soil conditions of the establishment site(s) to the genetic material available in the market is much easier today than in the previous years. This is due to written reports in journals and proceedings as well as more frequent visits by many to existing programs with plantations. The large assumption here is the experience level of the individuals making decisions of where to source seed for trials and plantations. Initial and subsequent germplasm introductions by seed, rooted cuttings, pollen or micropropagated plants must also consider the technical guidelines published by FAO (Ciesla et al., 1996).

The strategy of initial introductions should be to attain as wide a genetic base as possible. This base from the native range and established programs can be maintained in trials and pilot plantations for subsequent tree improvement and deployment strategies. However, initial operational plantations should use improved seed from the best source possible, matched to the soil and climate conditions of the area being planted. Use of seed from the native range for operational plantations will result in less volume production compared to plantations established with improved seed from selected sources.

Those organizations relying on purchased seed from identified sources would not technically be considered to have a tree improvement strategy. Only if their staff begin local collections to establish a land race will plantations be improved over the long term.

Improved Seed. Given that in the majority of sites for *E. grandis* and *E. urophylla* do produce seed it is not difficult to produce improved seed of these species. In many cases the seed is produced in thinned commercial stands or trials. It is also common to observe clonal seed orchards established with those individual trees that root from cuttings. Not many organizations, however, have established trials of seedlings to rogue orchards. Those organizations with more advanced programs tend to test clones for the roguing of seed orchards. Seedling progeny trials also allow the best families to be deployed.

Seed production in *E. grandis* and *E. urophylla* can quickly satisfy internal demand by an organization. This leads to the possibility to sell or make an interchange of seed with other areas. This ease of seed production has allowed much international movement of seed, some of good quality and some of poor quality. Also, many researchers or commercial forest managers do not place sufficient attention on the soils and climate of the site of origin of this seed before acquiring large quantities for planting. Significant losses in stand productivity result from using the wrong seed source.

Control pollination is used in some countries. The objectives are to produce new material for selection and to allow seedlings to be planted in trials to rogue seed orchards. However, many organizations do not have the technical skills to make successful control cross pollinations. Open pollination is a very well established method of tree improvement in eucalypts. However, the insect pollinated eucalypts do permit an accumulation of deleterious genes as was the case in the early years of eucalypt plantations in Brazil. Lack of pedigree control will limit future genetic improvement in programs relying on open pollination unless biotechnology techniques such as DNA fingerprinting can be utilized.

Cloning and Beyond. Following the stages of seed introductions and improved seed production those organizations seeking higher yields and more uniform wood will go to clones. Clones require more technical skill in nursery employees, silviculture and management. To be successful in clones requires a sustained financial commitment due to the costs of selection and testing. The most costly item in clones is the absolute requirement to test clones on multiple sites. With experience, clones matched to site will maximize yield.

Numerous systems have been developed for clonal production. Clones will have three to five times the cost of seedlings. The larger cost of clones will be due to the production and collection of coppice material from which to select cuttings for rooting. Early testing, tissue culture and other techniques can be used to reduce the time from selection of a superior individual from seedling origin to operational clonal plantations (Wright, 1995). It must be remembered that cloning is not tree improvement but rather the multiplication of superior phenotypes.

Tree Improvement. A wide range of tree improvement programs are underway for *E. grandis, E. urophylla* and urograndis. Most of them however have not reached the technical level that is now possible with these species.

Most large organizations have breeding populations or sub-lines that are established for specific site or product requirements. Advanced tree improvement programs use control cross pollination within specific breeding populations or sub-lines. Control cross progeny will be tested as clones or as seedlings. Pedigree control is more likely to be practiced today than in the past. While not covered in this paper, clonal testing strategies do interact with tree improvement programs. In fact the best programs decide a tree improvement strategy that includes clonal testing.

Efforts to rapidly derive seed from selected individuals of eucalypts are enhanced by hormone treatments including paclobutrazol. It is now feasible for organizations with modest budgets to produce seed and to make control crosses.

Species, Provenance and Hybrid Diversification. A small number of species, possibly as few as five, account for the majority of planting within the eucalypt genus which has 600 species. However, most organizations planting eucalypts have established trials or pilot plantations with a number of other eucalypt species. This activity must be continued and supported by management. Diversification is where future genetic gain will be made. In certain of the eucalypt species there is substantial variation between provenances and testing should include various provenances from a given species.

Another reason for planting more than one species is to be positioned for changes in market requirements. Currently in Brazil there is an effort to increase the quantity of sawn lumber recovered from eucalypts and this may include new species, provenances or hybrid combinations to reduce splitting prior to sawing.

Should climate changes achieve predicted levels in the future it is probable that many of the species in use today will have to be modified. Already certain companies are planting new species based on their perception of likely changes in temperature and rainfall in their land holdings.

Gene conservation is important for eucalypt species which, in some instances, are under pressure from land conversion to other uses. Those organizations with diverse plantings will have guaranteed access to the germplasm that they own. International efforts are underway by organizations like CAMCORE to collect seed of *E. urophylla* in Indonesia for gene conservation and eventual incorporation into tree improvement programs.

LESSONS FOR OTHER HARDWOOD PLANTATIONS

Initial plantation efforts with *E. grandis, E. urophylla* and urograndis were quickly successful and have led to more developed programs today. However there have been several key elements in the present and future success and these include:

1. Testing numerous seed sources i.e. species, provenances, families
2. Use of appropriate silviculture
3. Desirable wood for processing
4. Ability to produce seed at an early age
5. Coppice and rooted cutting for cloning
6. Ability to tissue culture or micro-propagate
7. Knowledge of disease and insect pests

CONCLUSIONS

There is considerable effort worldwide to improve *E. grandis, E. urophylla* and urograndis. Some of the difficulties in the years ahead will be a decreased genetic base in these species' native range due to logging, conversion to agriculture or other uses such as housing estates. One aspect that must be considered is access to genetic material in the native range by those organizations willing to participate in *ex-situ* gene conservation efforts especially when led by organizations such as CAMCORE. One lesson from agriculture is that permanent access to genetic material from the native range is essential for long term success in any plant genetic improvement program.

Numerous opportunities exist to obtain more genetic gain from these species. Improvements in the rooted cutting process (Wright, 1995) and in more sophisticated tree improvement strategies (Osorio et al., 1995) will continue. The use of biotechnology and marker aided selection hold much promise though how this technique can resolve genotype environment interactions is not clear. Infusion of genetic material be it seed, clones or strands of DNA will assist many organizations. Finally use of novel methods for incorporation of pulp and paper properties in a selection index will result in large economic gains.

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EXPRESSION AND STABILITY OF TRANSGENES IN ASPEN-*POPULUS*

M.R. Ahuja and M. Fladung

Abstract:-- We have employed *Populus* as a model system to investigate questions regarding stability and expression of foreign genes in transgenic trees. Several clones of European aspen (*P. tremula*) and hybrid aspen (*P. tremula* x *P. tremuloides*) were genetically transformed, using *Agrobacterium* as a vector system, with different gene constructs, in particular *rolC*. In order to test the expressive control of promoters, we have employed two types promoters: 35S from cauliflower mosaic virus, and light-inducible rbcS from potato, for control of *rolC* gene expression in transgenic aspens. The 35S-*rolC* chimeric gene caused marked alterations in growth characters, including stem height, internode length, leaf size and pale-green leaf color, and physiology in the transgenic aspens, as compared to untransformed controls. On the other hand, rbcS-*rolC* recombinant gene caused only minor alterations in leaf size, and the leaf color was similar to 35S-*rolC* transgenic aspens. However, in the second and third years growth cycles, the leaf color was somewhat similar to the controls. During the three year growth, deviations from the 35S-*rolC* syndrome, including leaf abnormalities, chimeras, and revertants to normal state were observed in the transgenic aspens. In order to study position effect on *rolC,* a heterologous transposable element *Ac* from maize was introduced into aspens along with the *rolC* gene, under the expressive control of 35S and rbcS promoters. Transgenic aspens carrying 35S-*Ac-rolC* and rbcS-*Ac-rolC* were morphologically similar to untransformed controls, because in the presence of *Ac*, the expression of *rolC* is inhibited due to position effect. However, following *Ac* excision during leaf development, there is restoration of *rolC* expression in the form of pale-green spots on the green leaf background. The presence of transgene was confirmed by PCR amplification of *rolC* and *Ac* coding sequences, and the copy number determined by the Southern hybridization, and gene expression was determined by northern blot analysis. These observations suggest that type of promoter/construct seems to affect growth, development and physiology of the transgenic aspens conditioned by the *rolC* gene. It also appears that *rolC* expression is variable during growth cycles.

Keywords: *Populus*, *rolC*, transgenic aspen, phenotypic alterations, gene expression

INTRODUCTION

Genetic engineering seems to offer prospects for forest tree improvement at an accelerated rate in plants. As compared to annual plants, forest trees have long generations cycles, with vegetative phases extending from one to several decades, and therefore may require special considerations for genetic manipulation. Therefore, it is relevant to ask if foreign genes would be stably integrated and expressed in the forest trees on a short-term or long-termbasis (Ahuja, 1988a, 1988b). Genetic stability of transgenic trees is important for their subsequent utility in the commercial forestry.

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Recent studies with several annual model plant systems have revealed that transgene expression may be variable and unpredictable (see Finnegan and McElroy, 1994; Pazkowski, 1994; Flavell et al. 1995; Meyer, 1995). Therefore, it is important to find out how model promoters and coding genetic sequences from other totally unrelated organisms are stably integrated in the genome and expressed at the biochemical/molecular and phenotypic levels in forest trees.

We have employed *Populus* as a model system to address these and related questions. Poplars and aspens (*Populus* spp.) can be relatively easily regenerated in tissue culture (Ahuja, 1986, 1987, 1993; Chun, 1993; Ernst, 1993) and can be genetically transformed using the *Agrobacterium*-vector system (Fillatti, et. al. 1987; Klopfenstein et al. 1993; Confalonieri et al. 1994; Fladung et al. 1997b).

MATERIALS AND METHODS

By employing a leaf disc co-cultivation methodology (Fladung et al. 1997b), three clones of European aspen (*Populus tremula*) and one of hybrid aspen (*P. tremula* x *P. tremuloides*) were genetically transformed by *Agrobacterium*-mediated binary vector system. A number of gene constructs, in particular those carrying the *rolC* gene from *A. rhizogenes*, were included in this study. *RolC* is a dominant pleiotropic gene, which affects plant growth, phenotype, and physiology of transgenic plants. Of course, the phenotypic effects of the *rolC* gene are different, depending upon its heterologous promoter. Two types of promoters were include in the study to monitor *rolC* expression in the transgenic aspens. These included a 35S promoter from Cauliflower mosaic virus, and light-inducible rbcS promoter from potato. In addition to the *rolC* chimeric gene, the construct also carried a linked gene for kanamycin resistance (*npt II*), which served as a selectable marker for screening putative transformants (Fladung et al. 1997b).

Methods for DNA and RNA isolation from the leaf tissues for the PCR and Southern blot hybridization have been previously described (Fladung and Ahuja, 1995, Faldung et al. 1997b).

RESULTS AND DISCUSSION

Phenotypes of transgenic plants. Several thousand transformants have been produced in tissue culture and maintained on a kanamycin-containing medium in the growth chambers. From these, more than 1500 transgenic aspens have been grown in the greenhouse during the past three years to investigate transgene expression and stability (Ahuja and Fladung, 1996; Fladung et al. 1996, 1997a, 1997b,). During the first year of growth, transgenic aspens carrying 35S-*rolC* gene much smaller leaves, compacted internode, and the stem was relatively reduced in height, as compared to untransformed controls. On the other hand, transgenic aspens carrying the rbcS-*rolC* gene exhibited slightly smaller leaves, as compared to controls. But their stem height and internode lengths were rather similar to controls. One trait shared by the 35S-*rolC* and rbcS-*rolC* transgenic aspens was the pale-green leaf color, as compared to green leaf color in the untrasformed controls. However, during the second and third year growth cycles, the leaf color in the transgenic aspens after flushing were pale-green, but later on the leaf color turned green as is in the untransformed controls.

In addition to phenotypic alterations in the transgenic aspens, there were also changes in the hormone metabolism. Hormone levels were different in transgenic conditioned by 35S-*rolC* and rbcS-*rolC*, and untransformed controls (Fladung et al. 1997a). In particular, the 35S-*rolC* transgenic aspens showed significantly lower levels of abscisic acid (ABA) at the predormant and dormant bud stages in fall, as compared to controls of similar age. The 35S-*rolC* transgenic aspens showed flushing at least two weeks earlier than controls in spring (Fladung et al. 1996, 1997a), suggesting possible involvement of ABA in the flushing process.

During the three year growth cycles, deviations from the typical *rolC* phenotype, in particular 35S-*rolC* transgenic aspens were observed. These included leaf abnormalities, chimeras, and revertants to normal state. Some of these phenotypic changes may be due to epigenetic events, possibly involving gene inactivation due to methylation, while others could be due to impairment or loss of the transgene. We are in the process of examining the mechanisms of transgene inactivation.

In order to study position effect on the *rolC* gene, a transposable element *Ac* from maize was inserted between the *rolC* gene and the promoter. In one gene construct, both *Ac* and *rolC* genes were regulated by the 35S promoter, while in the second construct these gene were controlled by the rbcS promoter. Transgenic aspens carrying the 35S-*Ac-rolC* or rbcS-*Ac-rolC* chimeric genes were morphologically similar to untransformed controls, in terms of leaf size and green color, internode length, and stem height. However, following *Ac* excision during leaf development, there is a restoration of *rolC* expression in the form of pale-green spots on the green leaf background (Fladung et al. 1977b). There was variation in the size and shape of pale-green sectors in different transgenic clones. These pale-green spots on the leaves are apparently caused by the excision of *Ac* from its original position in the recombinant gene in the periclinal chimeric leaves of 35S*Ac-rolC* transgenic aspen (Fladung and Ahuja, 1997).

Molecular characterization. Most putative transgenic aspen clones that exhibited the *rolC* phenotype also tested positive for the *rolC* gene by PCR analysis. However, the copy number of the *rolC* gene, as revealed by the Southern blot non-radioactive hybridization (Faldung and Ahuja, 1995) varied between one and three in the transgenic aspens (Ahuja and Faldung, 1996; Fladung et al. 1997b). The levels of *rolC* expression seemed to be affected by the copy number of the transgene. Those with more than one copy of the *rolC* gene were severely dwarfed, showed much smaller leaves, and seemed to be less viable.

Some of the revertant/chimeric shoots showed the presence of the *rolC* gene, while others exhibited its absence (Fladung et al. 1997b). Northern blot analysis of those revertants still carrying the *rolC* gene either showed the presence of the RNA transcript, or a lack of the transcript in those shoots. Whether, the transgene is inactivated in specific leaf layers, possibly due to the methylation of the promoter or the coding sequence is under study.

Most of the 35S-*Ac-rolC* transgenic aspens carried both the *rolC* and *Ac* coding sequences, as determined by the PCR analysis. However, at least, three clones which were phenotypically similar to untranformed aspen, carried only the *rolC* gene, but not the *Ac* element. Southern blot analysis showed the presence of one copy of the *rolC* gene and one of *Ac* in most of the 35S-*Ac-rolC* transgenic aspens. In the northern blot experiments, a specific *rolC* specific transcript was detected

only in the light-green sectors of the leaf , but not in the green areas of the leaf (Faldung and Ahuja, 1977).

CONCLUSIONS

These observations suggest that the type of promoter/construct controlling *rolC* expression seems to affect the growth and differentiation in transgenic aspens. It also appears that *rolC* expression is variable in transgenic aspens, as judged by the morphology, but the genetic basis of varaibility needs to be confirmed by molecular analysis.

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BREEDING STRATEGY FOR THE FIRST-GENERATION OF PINUS TAEDA IN THE NORTHEAST REGION OF ARGENTINA

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Abstract: A strategy for the Genetic Tree Improvement Cooperative of the northeast region of Argentina was developed to start with a broad genetic base to ensure maximum genetic gain in the long-term as well as provide insurance against new diseases or traits that may become important in the future. The breeding strategy for the first-generation is simple, cost-effective and yet as powerful as possible, using well-known technology for the species. The primary objective is to make near-optimal genetic gain in two traits (volume growth and straightness). At the same time, the strategy is flexible enough to permit each member to pursue traits of special interest. Also, it will provide for mixing and interchange of alleles among many sources and types of selections (different provenances, plantation selections, backward selections). The aim is to produce, in one or two generations of breeding, a well-adapted and genetically improved land race for Argentina that combines the best alleles from the many different types of selections and sources. The strategy relies on use of open-pollinated seed from each selection for the first-generation, but will permit open pollination or control pollination for breeding and testing in the second-generation. The main breeding population will be composed of 600 selections and sub-divided in twelve unrelated lines of 50 open-pollinated families in each line.

Keywords: Loblolly pine, breeding population, backward selection, forward selection, sublines.

INTRODUCTION

The Cooperative Forest Genetic Improvement Program of the Centro de Investigaciones y Experiencias Forestales (CIEF) began in 1984 with the genetic improvement of the two more widely planted conifers in the country. At that moment *Pinus elliottii var. elliottii* was the most planted species in the northeast region of Argentina. Nevertheless results from old trials proved and the new ones confirmed that *Pinus taeda* displays important provenance variation and that the best provenances (from Florida) were much more productive than *Pinus elliottii* especially in the more fertile red-argilic soils. Now *Pinus taeda* is preferred and more than 80% of the pines planted in this area is with *Pinus taeda.*

In 1986 a breeding program for *Pinus taeda* began with selections made from a plantation of an unimproved source from Marion-FL. In 1986 the first clonal seed orchard from this

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source and the first OP progeny tests were established. Other sources, such as Livingston Parish-LA, also proved to be superior in volume growth in relation to the unknown provenance commercial seedlots planted up to that moment. Over the past two years (1995-1996) more selections were made, not only in commercial plantations of Marion-FL, Columbia-FL, Livingston Parish-LA and other sources, but also in old provenance tests so as to screen all the local genetic base of the species.

Today the CIEF, in the northeast of Argentina, has six members whose main products are pulp and solid wood. Plantations were planted in the past years at densities of approximately 1800 trees per hectare and managed for a 20 to 25 year rotations. In 1996 the *Sirex noctilio* was detected for the first time in some plantations and the density was lowered to 1400 to 1100 trees per hectare as a sanitary decision. Commercial thinning is a normal practice with commonly two over the rotation. The mean annual increment ranges from 18 m^3/ha.-yr to 28 m^3/ha.-yr in the more fertile sites. The plantation area operated by the associated members is approximately 60,000 ha located in the northeast of Argentina (provinces of Misiones and Corrientes). In the last two years new companies in the region have raised annual forestation to 20,000 ha. Most of the seed utilized to establish these commercial plantations has come from clonal seed orchards from the United States (Marion and Livingston sources) and a little is produced by a local seed production area from Marion-FL provenance. The first clonal seed orchard (Marion clones) began to produce seed for operational plantation this year.

This paper describes the process to establish the first-generation breeding population for the CIEF.

BIOLOGICAL, GENETIC, AND ORGANIZATIONAL PREMISES

Biological Characteristics

Most trees of *Pinus taeda* are producing flowers by age six years in the northeast region of Argentina. Flowering and pollination occur in June and July and cones ripen in March and April. *Pinus taeda* grafts easily and all clones flower five years after the establishment of the orchards with estimated full seed production by age 10. All propagules for operational plantations are currently seedlings. Control pollination is a well-known technology with *Pinus taeda* for use in breeding programs. *P.taeda* has been a recalcitrant species in terms of vegetative propagation. There are few if any company in the world using vegetative propagules (such as rooted cuttings or tissue culture plantlets) on an operational basis. As, with most of conifers, *P.taeda* suffers inbreeding depression (Bridgwater and Franklin, 1985; White et.al., 1986). Thus inbreeding will be avoided in seed orchard seed destined for operational plantations.

Genetics premises

Differences among provenances are very important in *Pinus taeda.* In the northeast of Argentina as well as in other sub-tropical climates (such as southern Brazil, Zimbabwe and

South Africa), provenances from Florida grow fastest. In Argentina, the Marion county source has been widely planted with good success, but Marion is only one of many Florida sources that would do well in Argentina. Further, the Livingston Parish source does well in the region. Thus, the first-generation breeding population should include a wide variety of material from the southern and coastal areas of the *Pinus taeda* natural range with emphasis on the Florida provenances.

Many of the existing plantations of loblolly pine in Argentina came from unknown provenances in the US. Further, most plantations originated from sub-optimal provenances because the Florida sources have only recently been used. Therefore, both to increase the genetic base and to increase genetic gains it will be necessary to screen all the local genetic resource (in local provenance trials) and infuse external genetic material from the US or other parts of the world.

Genetic variation among families within each provenance is also a very important source of genetic variation in *Pinus taeda*. The heritability for volume growth is in the range of 0.15 to 0.20, while that for straightness is between 0.25 to 0.35. The two most important traits for the cooperative breeding program are volume growth and stem straightness. Other traits as wood density and branch quality are being considered and probably will be given emphasis with different stress in each members' breeding program depending on the final products.

Fusiform rust has not been detected in Argentina. No species of *Quercus* is planted commercially in the area, nevertheless some trees are occasionally grown in public areas and parks. It seems very unlikely that the disease will become important in the region, but the breeding population will have a wide range of resistant and susceptible genotypes. So it would be possible to breed for resistance.

Some genotype x environmental (g x e) interaction exists within the *P.taeda* planted range of northern Argentina if we consider the extreme soil types more fertile and deep soils in the north and more shallow soils in the south of the area. Some early results from progeny tests support this idea (Bunse et.al. 1992).

The CIEF breeding strategy will rely on recurrent selection for general combining ability. The appropriate age to make selections depends on many factors. In the current mass selection from local plantations ages more than seven years were preferred. For progeny tests, six to seven years seems like the best age to rank families, make within-family selections and use the data to rouge seeds orchards. Measurements from these ages should be taken before the first thinning. Evidence indicates that family rankings after these ages should be stable with high juvenile-mature correlation after age seven (Lowe and van Buijtenen, 1989). Also this age of seven years-old is one-third to one-half of the final rotation. In the future, new information from the progeny tests being established may indicate that an earlier age is better.

Organizational Decisions

These decisions were made jointly by the associated members of this breeding program: 1. The breeding strategy for the first-generation should be as simple, cost-effective and yet powerful as possible, using well-known technology for the species. 2. The primary objective is to make near-optimal genetic gain in the most important traits. The major characteristics for breeding are volume growth and straightness; but the first generation strategy must be flexible enough to permit each member to pursue traits of special own interest. 3. All breeding and testing efforts will be conducted cooperatively with free exchange of genetic material among members. 4. The production population (seed orchards in the first-generation) for commercial production of propagules will be developed individually by each member. 5. The strategy will maximize gain for clonal seed orchards as this is the primary type of production population.

FORMATION, COMPOSITION, STRUCTURE AND SIZE OF THE FIRST-GENERATION BREEDING POPULATION

Formation of the main population

The material potentially available for inclusion in the main-breeding population consists of:

- Selection from genetic resources already present in Argentina. This includes: 1. Selections in commercial plantations from age seven to age twenty-five (rotation-age). 2. Backwards selection from selected trees from 1984 to 1992. 3. Forward selections from new progeny tests whose parents are not present in the country.

- Selections from a new seed collection from the native range. This collection will be made probably in 1997 or 1998. The only requirement will be that the trees been healthy and dominant growing in wild stands from the southern area of the native range.

Composition

The first-generation main breeding population will consist of 600 OP-families established at up to nine test locations (**Figure 1**). Half of the families will be from local selection and the other half from the new US collection. The final composition of the main breeding population will have a clear emphasis on the Florida and Livingston Parish sources (70%) with lesser representation of the local unknown provenance (20%), other American provenances (5%) and Zimbabwean and South African (5%) provenances.

Structure and size

The main breeding population of 600 selections is sub-divided into 12 sublines of 50 OP-families in each subline. Each subline should be formed with 50 OP-families from a variety of sources with the intent of starting with 12 genetically-equivalent sublines. That is, each

subline will have 50 different OP-families sampled systematically, with a few families from each source, to ensure that the mean genetic values of the twelve sublines are similiar. This will allow gene mixing in each subline meaning that each subline will have the potential to develop into a well-adapted and genetically-improved land race (**Figure 1**).

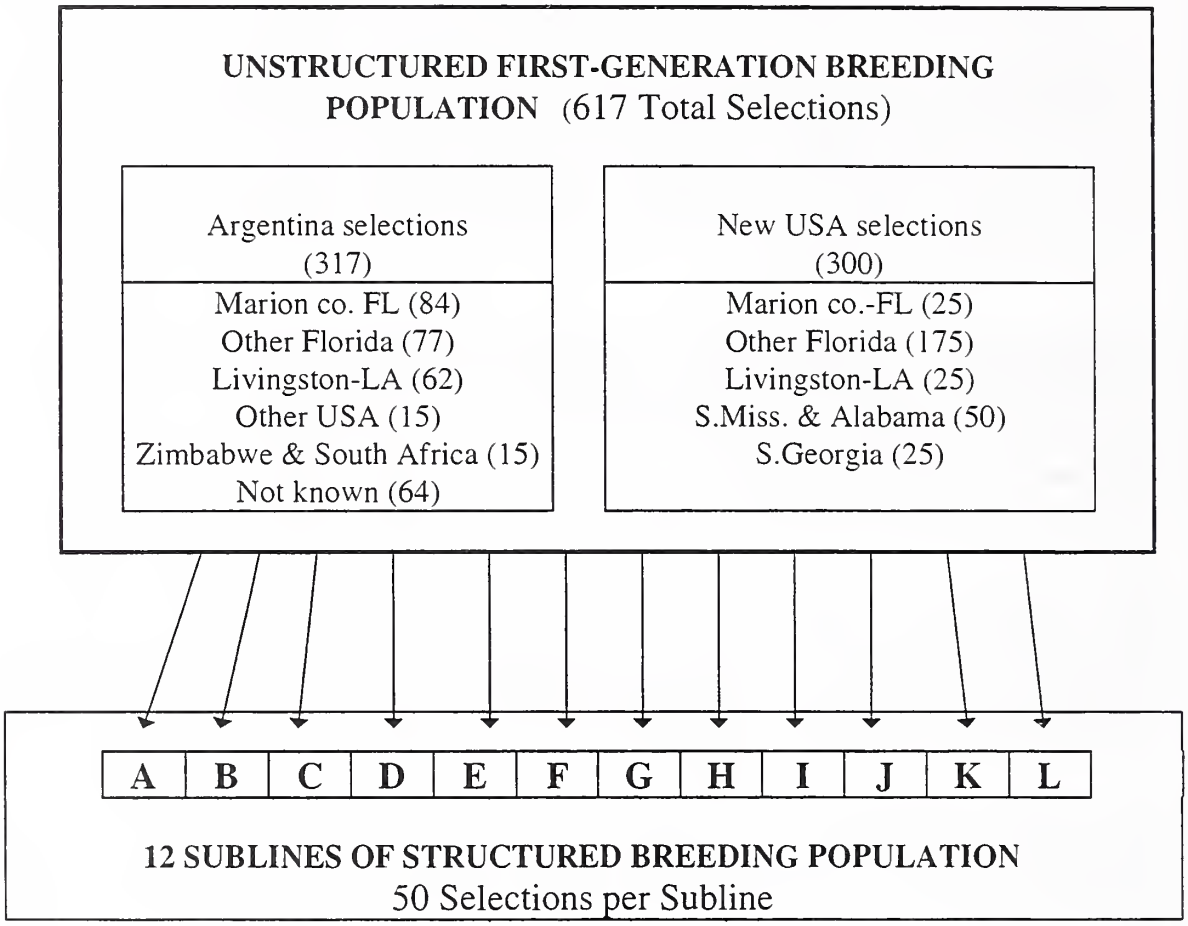

Figure 1. The first-generation breeding population of 600 OP families is subdivided into 12 sublines of 50 OP families in each subline. The goal is to mix genes from all provenances and types of selections by forming each subline with a few families from each source.

For members planning on using wind-pollinated clonal seed orchards as the primary type of production population, twelve sublines is an appropriate number for a strategy that will relay on OP seed orchards. In future generations, two members of each sublines could be selected to compose the seed-orchard ensuring at least 30 m separating among relatives (i.e., ramets of a clone or relatives from the same subline) (Lowe and van Buijtenen, 1986; White, 1992). Further, the size of each subline (50 OP-families) is large enough to ensure good genetic

gains for at least 10 generations of breeding (Kang, 1979; Mahalovich and Bridgwater, 1989; review by White 1992).

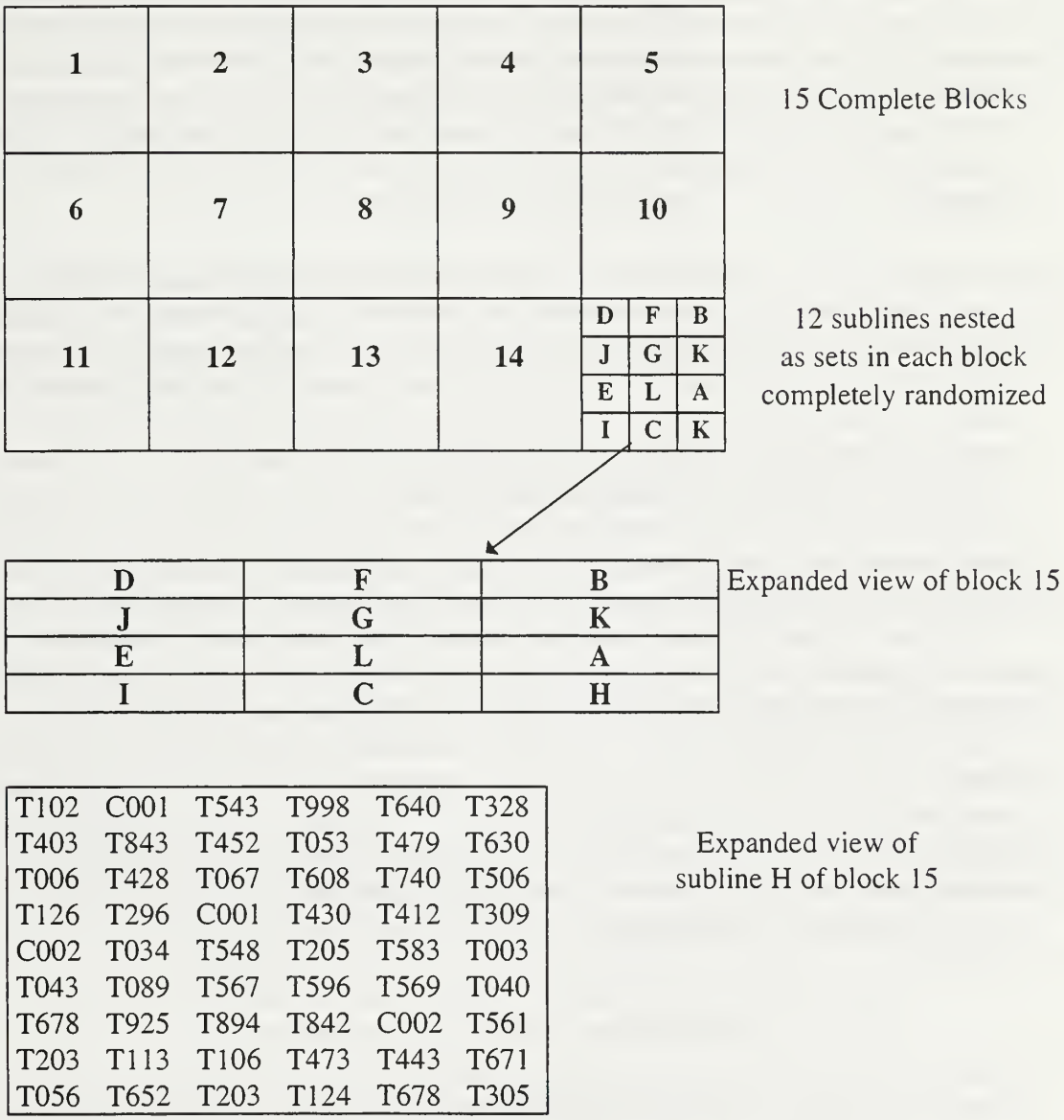

Figure 2. Diagram of one *Pinus taeda* first-generation progeny test containing 600 OP families. There are 15 complete blocks with each block containing the 12 sublines (A, B, ... L) nested as sets randomly placed within each block. Each set is composed of 54 seedling planted randomly: one seedling from each of the 50 OP-families forming that subline and 2 seedlings from each of the two checklots. In total there are 9,720 measured trees and approximately 1,300 buffer trees per location for a total area of nearly 7 ha.

ESTABLISHMENT OF THE MAIN POPULATION

The main breeding population of 600 OP-families will be planted and managed as OP-families for the first generation of breeding. In addition to the theoretical considerations, use of 12 sublines is convenient logistically: the 3 bigger companies will physically manage 2 lines each and the 3 smaller members 1 line each. Because the main population is sub-divided into 12 sublines, it is desirable to have maximum statistical efficiency in the ranking of the 50 OP families within a given subline, even if this means that the ranking between sublines has slightly less precision. The field design of each test is a randomized complete block design with sets of 50 OP-families nested within blocks and single tree plots. Each location will contain the entire first-generation breeding population of 600 OP-families (**Figure 2**).

Only one series of genetic tests will be established and many objectives will be accomplished with it: 1. To rank the 600 families to permit rouging of seed orchards, establishment of 1.5 generation seed orchard with only the best clones and deployment of specific families best suited to specific sites and products. The large number of sites is needed for these objectives. 2. To describe the genetic structure and estimate important genetic parameters in the region: (i) heritability for all important traits; (ii) importance of g x e interaction; (iii) genetic correlations among traits; and (iv) juvenile-mature correlations to determine optimal selection age. All these parameters will be useful when designing the second-generation breeding strategy. 3. To rank the families to determine which should be eliminated from the second-generation breeding population due to their genetic inferiority. 4. To provide a source of genetic material in which to make forward selections for both the second-generation main population and the second-generation elite population and 5. To maintain a broad genetic base. There will be 600 families and 81,000 different genotypes which is a large genetic base. 6. To allow conversion of genetic tests into seedling seed orchards capable of producing commercial quantities of seed with substantial genetic improvement after second and third rougings. 7. To estimate genetic gains from the first-generation of the tree improvement in the northeast region of Argentina. This will be accomplished through inclusion of two checklots that will serve as the base or reference population against which future gains will be measured and quantified.

GENERATION INTERVAL AND WORKLOAD

Under the strategy described, we shall be able to complete the first generation in 8 years (from 1998 to 2006) and two years more for seed collection, sowing and planting the second generation. This seems an efficient period using a low cost alternative (relying on OP families to manage the breeding population) and a selection age of seven years.

In terms of loadwork it also seems reasonable in view of the actual capability of the members of the cooperative, but also allows flexibility to increase the breeding effort if the planted area enlarges in the future.

ELITE POPULATION AND SECOND-GENERATION BREEDING STRATEGY

Considering the fact that most members plan to rely on OP seed orchards to produce seed for commercial plantations, the use of an elite population will be reviewed when some techniques such as control pollination and / or vegetative propagation for mass production of plantlets for commercial plantation will be better developed among the members. We expect that by the time we arrive at the second-generation, these techniques will be more developed in our cooperative and the exact strategy will depend on the level of technology achieved.

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WHITE PINE CONE BEETLE POPULATION TRENDS IN NORTH CAROLINA AND TENNESSEE SEED ORCHARDS 1986 – 1997

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ABSTRACT

Overwintering white pine cone beetle (WPCB) populations within dead infested cones were sampled from six eastern white pine seed orchards beginning in 1986 and continuing through 1997. Two sampling systems were used on each orchard: 1) 50 Ft.^{2} Sampling System, and 2) Random Sample System.

Beetle populations peaked on the Edwards Seed Orchard in 1992 and 1995 while populations in most Tennessee orchards peaked in 1992 – 1993 and 1995. In comparison population peaks were noted on the Beech Creek in 1988, 1993 and 1997. During the intervening years, populations were moderate or crashed. Beetles populations were low on the Beech Creek or Edwards Orchards in 1994 while slightly higher populations were present in the Tennessee orchards. These data suggest that in Tennessee during 1994, beetle populations were increasing. The 1995 survey data indicated high populations on the Pickett at 76.6 live beetles per 50 ft^2 sample. This was the largest overwintering beetle population recorded during the survey. Populations of WPCB were also high at the Norris and Stephens orchards. In 1996 populations crashed.

In most years, the Picket Seed Orchard in Tennessee had higher sustained overwintering WPCB populations than all other orchards. In comparison, the Edwards Seed Orchard generally had the fewest overwintering beetles. Comparisons between sampling techniques indicate the 50 ft^2 method was easier to use for evaluating population trends. No cone crop damage data were collected during many of the sample years, thus no analysis or correlation between cone crop damage and overwintering WPCB populations were possible. However, we observed that few cones were killed when populations were low, but losses approached 100% for high populations without protection.

KEYWORDS: Eastern white pine, white pine cone beetle, seed orchard, *Conophthorus coniperda* (Schwarz), *Pinus strobus* L.

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INTRODUCTION

White pine, *Pinups strobes* L., seed orchards throughout the Eastern United States are continually under attack by an array of insect pests. The most significant pest is the white pine cone beetle (WPCB), *Conophthorus coniperda* (Schwartz) which often causes extensive losses to cone crops. Historically liquid applications of insecticides such as DDT and others have almost always failed (DeBarr et. al 1982). During the 1970's and 1980's carbofuran was registered and used to control this pest but the registration was withdrawn in the early 1990's. Recent tests with Asana XL®, esfenvalerate, indicate that it is effective in WPCB damage reduction (DeBarr and Barber unpublished). The use of prescription fires in late winter to kill overwintering beetles is effective and is used in many white pine orchards (Wade et al. 1989).

A method to predict destructive overwintering white pine cone beetle (WPCB) populations is necessary to effectively manage white pine orchards. During periods when beetle populations are low, it may be possible to not treat the orchard thus reaping economic or environmental benefits from not spraying or burning. During high WPCB populations, all necessary steps to control the beetle may be warranted.

LIFE CYCLE

Beetles emerge around the first of April in western North Carolina, fly into the treetops and attack developing cones. The beetles bore into the bases of cones at the junction of the petiole and produce a characteristic entrance hole surrounded by a circular mass of resin-soaked frass. Each beetle can attack and kill up to 4 or 5 cones. Mating and egg laying occurs in the attacked cone. The larvae develop in the infested cone and become adults in mid summer. During the summer and fall most of the infested cones fall to the ground where the beetles overwinter.

MATERIALS AND METHODS

There were two sampling systems utilized to measure overwintering beetle populations.

1. Random Sampling System – This system involves walking across the orchard and collecting 150 cones from the top of the orchard duff. The cones collected were ones that were infested and killed the previous year by the WPCB. Of the 150 cones collected, 100 intact cones of varying sizes were dissected and the number of live and dead beetles determined.

2. 50 Ft^2 System – This system involves pre-selecting 20 trees and randomly determining a side of the tree to collect from. After a 5 ft. by 10 ft. area is selected and marked off with string all the cones are removed for later dissection. The cones were dissected and the number of live and dead cone beetles determined.

All white pine seed orchards in North Carolina and Tennessee were sampled during January or February during each year of this survey. The orchards listed by agency are as follows: 1) USDA Forest Service, Beech Creek Seed Orchard, Murphy, NC, 2) North Carolina Forest Resources , Edwards Seed Orchard, Morganton, NC, and 3) Tennessee Division of Forestry, Norris Seed Orchard , Norris TN, Knoxville Seed Orchard - Knoxville, TN, Stevens Seed Orchard – Oak Ridge TN, and Picket Seed Orchard – Jamestown, TN.

RESULTS AND DISCUSSION

In Figures 1-5, data are displayed that show the seasonal fluctuations of overwintering WPCB populations at eight seed orchards in Tennessee and North Carolina. On the Beech Creek, populations were higher during 1988, 1993 and 1997. Beetle populations were the lowest from 1990 – 1992 and again in 1994 - 1995. While both sampling methods detect peak populations, the 50 ft^2. sampling method more clearly identifies the two peaks when both sampling methods were used simultaneously.

On the North Carolina Division of Forest Resources' Edwards Seed Orchard located in Morganton, NC, beetle populations peaked during 1992 and 1995. These data are difficult to interpret because of conflicts between yearly totals among sampling systems. Overall, the peak populations detected were less than found on the Beech Creek or most Tennessee orchards.

Data from the Stephens, Norris, and Pickett Seed Orchards in Tennessee all indicate peak populations in 1992 and 1995. In contrast, data from the Knoxville orchard indicate a peak in 1993. In 1997 the 50 ft^2. System indicated an increasing population while data from the random sample showed only a slight increase over the previous year. Generally beetle populations at the Pickett, Norris and Stephens orchards reached peak populations that were higher than recorded for the other five orchards of the survey.

Unfortunately, annual cone crop loss data was not collected during most of this period. We were therefore, unable to analyze the data to determine thresholds for economic damage or correlation's between overwintering WPCB populations and damage. We have observed, however, that low WPCB populations are usually responsible for minimal cone losses and that conversely high populations such as found on the Beech Creek in 1997 result in nearly total crop losses if untreated.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the staff of the Beech Creek Seed Orchard for their help and assistance collecting field data over the period of this evaluation. Also, appreciation is expressed to those individuals with the Tennessee Division of Forestry and the North Carolina Division Forest Resources for their help and assistance with this work.

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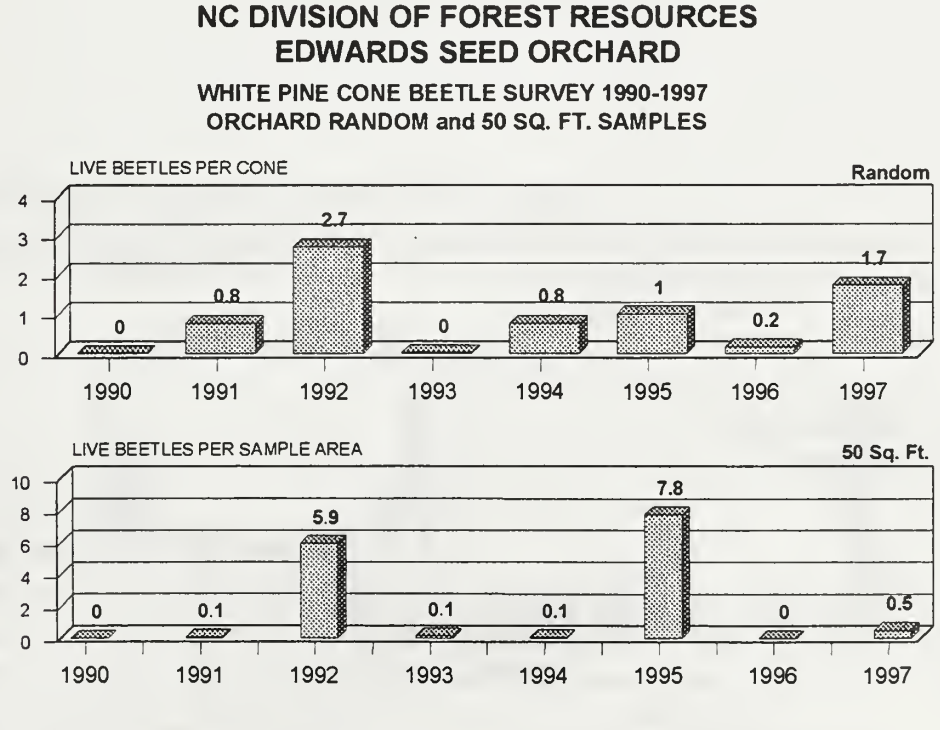
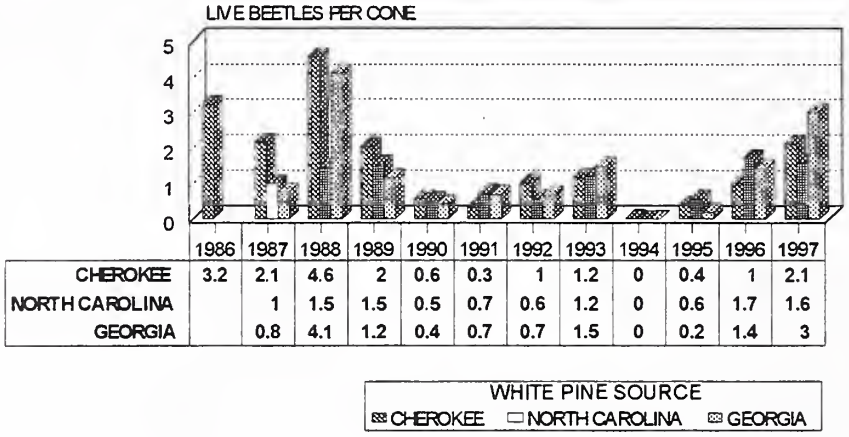


Figure 1. Random and 50 Sq. Ft. sampling data from the NC Division of Forest Resources

USDA FOREST SERVICE - BEECH CREEK SEED ORCHARD

WHITE PINE CONE BEETLE SURVEY 1986 - 1997
RANDOM SAMPLE

Figure 2. Random sampling data for the Beech Creek Orchard



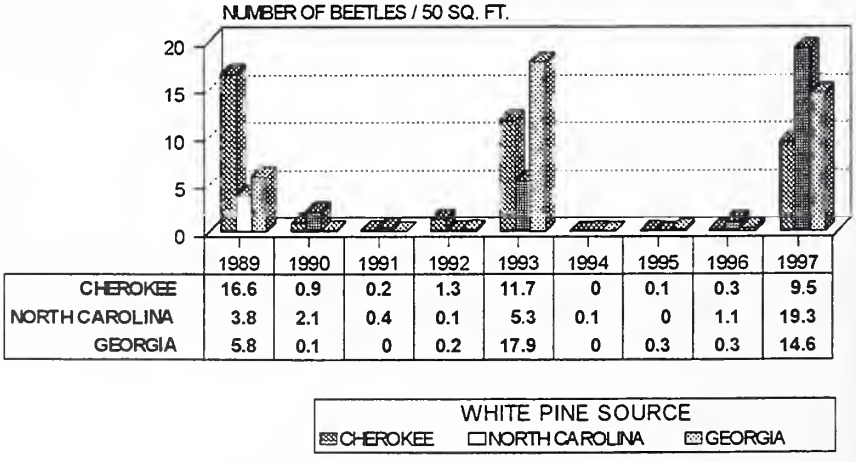
	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997
CHEROKEE	3.2	2.1	4.6	2	0.6	0.3	1	1.2	0	0.4	1	2.1
NORTH CAROLINA		1	1.5	1.5	0.5	0.7	0.6	1.2	0	0.6	1.7	1.6
GEORGIA		0.8	4.1	1.2	0.4	0.7	0.7	1.5	0	0.2	1.4	3

USDA Forest Service, Tusquitee Ranger District, Murphy, NC
Surveys made between January and March of each year.

USDA FOREST SERVICE - BEECH CREEK SEED ORCHARD

WHITE PINE CONE BEETLE SURVEY 1989 - 1997
50 SQ. FT. SAMPLE

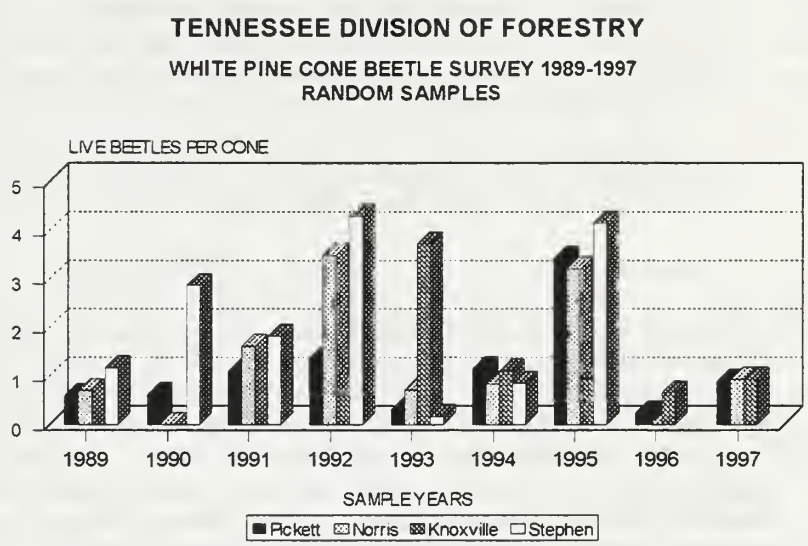
Figure 3. 50 Sq. Ft. sampling data for the Beech Creek Orchard



	1989	1990	1991	1992	1993	1994	1995	1996	1997
CHEROKEE	16.6	0.9	0.2	1.3	11.7	0	0.1	0.3	9.5
NORTH CAROLINA	3.8	2.1	0.4	0.1	5.3	0.1	0	1.1	19.3
GEORGIA	5.8	0.1	0	0.2	17.9	0	0.3	0.3	14.6

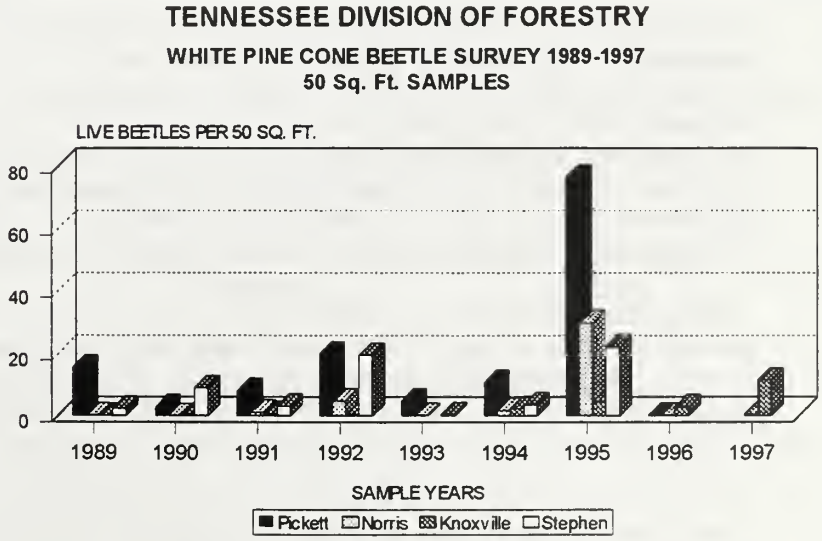
USDA Forest Service, Tusquitee Ranger District, Murphy, NC
Surveys made between January and March of each year.

Figure 4. Random sampling data for Tennessee Orchards



Surveys made between January and March of each year.
Stephens Switch Orchard was dropped from the survey in 1996.

Figure 5. 50 Sq. Ft. Sampling Data for Tennessee Orchards



Surveys made between January and March of each year.
Stephens Switch Orchard was dropped from the survey in 1996.

IMPROVING THE QUALITY OF LONGLEAF PINE SEEDS FROM ORCHARDS

James P. Barnett[1]

Abstract.--Longleaf pine (Pinus palustris Mill.) seeds are sensitive to damage during collecting, processing, storing, and treating activities. High quality seeds are essential for successful regeneration of the species by either direct seeding or planting. Results from recent tests are combined with earlier data to develop recommendations for producing and maintaining longleaf pine seeds of high quality.

Keywords: Pinus palustris, southern pines, nursery production, germination, cone and seed production.

INTRODUCTION

Longleaf pine (Pinus palustris Mill.) is a highly desired pine species for reforestation in the southern Coastal Plain of the United States. Vast acreages of virgin longleaf pine previously existed across the South from eastern Texas to North Carolina. However, the species is characterized by a lack of regeneration on sites with extensive amounts of competing vegetation. Longleaf pine has no early epicotyl growth, and its peculiar "grass stage" contributes to its sensitivity to competition and brown-spot needle blight (Mycosphaerella dearnessii Barr.).

Regeneration of longleaf pine has become more difficult with the advent of fire control, and longleaf has failed to maintain its competitive position because other southern pine species are relatively easier to regenerate. Southwide acreage in longleaf pine is now less than 10 percent of that in the original forests. However, interest in longleaf pine is increasing because it resists insects and diseases, produces high quality solid-wood forest products and is an unique ecosystem.

An essential element to improving reforestation success is increasing the quality of longleaf pine planting stock. A number of nursery cultural treatments can be used to improve the quality of seedlings (Barnett 1990, Shipman 1958, Shoulders 1963, Wakeley 1954), but the key to seedling quality is uniform germination and early establishment in the nursery. Developing a uniform nursery crop depends upon the availability of high quality seeds. Cultural practices, either in container or bareroot nurseries, cannot effectively overcome the problems resulting from inadequate germination.

Longleaf pine seeds are the most difficult of the southern pines to collect, process, store, and treat successfully (Wakeley 1954, Barnett and Pesacreta 1993). Because the seeds are large, have thin seedcoats, and are unusually moist when extracted from cones, collecting and processing them without

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adversely affecting quality requires special handling and unique procedures. Producing longleaf pine seeds to meet the increasing demand in recent years has been plagued by low seed quality. This paper presents results from recent tests, combines these results with other documented findings, and develops recommendations that may improve the potential to produce high quality longleaf pine seeds from our southern pine orchards.

COLLECTING SEEDS

The greatest early losses in seed quality result from collecting cones before seeds are fully mature. Generally, timing southern pine cone collection is based on Wakeley's (1954) results that indicate cones are mature enough for seed extraction when their specific gravities drop below 0.89. More recent data confirm that collection should be delayed until cones are fully mature, because viability of longleaf seeds from immature cones may decrease during cone storage (Barnett 1976a, McLemore 1975a) if some undetermined stage of ripeness has not been reached (Barnett and Pesacreta 1993).

Tests were conducted in fall 1994 to determine where major losses in seed quality were occurring. Specific gravity (SG) was measured on cones from several clones that were collected on an operational basis in the Stuart Seed Orchard at Pollock, LA. The cones were collected during two collection periods and both lots were divided for shipment to two commerical seed processing plants. Collection 1 (October 5-6) was delayed until average cone specific gravity was below the level that indicated maturity (table 1). In collection 2 (October 20), cone SG was lower. It is important to note that even with an average SG of 0.86, a large portion of the cones had a much higher SG. Wakeley (1954) recommends that cone collection begin when **19 of 20 cones** have a SG of less than 0.89. The data from the 1994 tests indicated that average SG must be about 0.81 before Wakeley's criteria are met.

The data also confirmed the previously reported influence of SG on seed yields (figure 1); the lower the SG, the higher the seed yield (table 1). Seed germination was also markedly affected by cone maturity. Average germination of seeds from collection 1 was 51 percent compared to 69 percent for collection 2 (table 2).

Table 1. Longleaf pine cone specific gravity[1] and seed yields by collection date.

Collection period	Cone specific gravity			Seed yields
	Average	Above 0.89	Above 0.87	
		------percent-----		lbs/bu
1 (Oct. 5-6)	0.86	27	44	0.49
2 (Oct. 20)	.81	4	7	.73

[1]Values represent an average of 20 replications of 10 cones each per collection period.

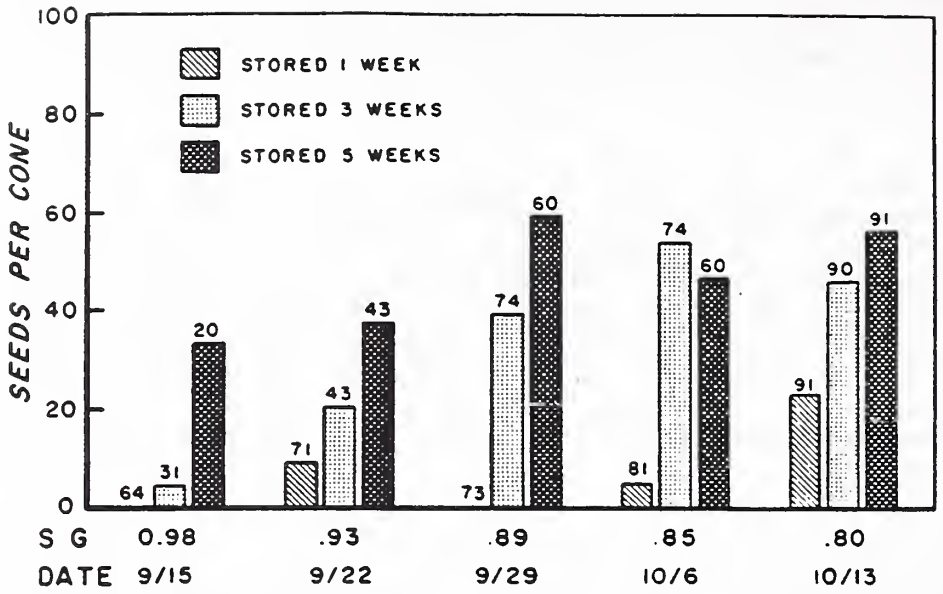

Figure 1. Seed yields and germination (shown above bars) of longleaf pine as affected by date of collection and cone storage (from Barnett 1979a).

Ripening immature or holding mature longleaf cones before extraction may or may not improve seed viability (Barnett 1976a, 1976b; Bonner 1987; McLemore 1959, 1975a), but some cone storage is needed to improve seed yields.

After SG's were measured, the cones were shipped to two processing plants. Dataloggers included in the bags of cones recorded hourly temperature exposures during cone storage and processing periods. Duplication of processing provided a greater range of environmental conditions and thus improved the opportunity to identify conditions that might affect seed quality. Table 2 provides a summation of cone and seed exposure.

Information collected on the dataloggers shows differences between processing plants in the length of time the cones were held before kilning and in the temperature exposures during kilning and seed drying (table 2). Delaying cone extraction beyond 30 days may begin to reduce seed quality (figure 2), but the effects of cone storage are difficult to separate from those of exposure to temperature. These two variables may interact. For instance, longer cone storage could improve seed germination (Bonner 1987), but the corresponding longer exposures to high seed drying temperatures might reduce viability.

PROCESSING SEEDS

During the processing stage, dewinging may adversely affect the quality of seeds collected from mature cones. During our 1994 operational tests, dewinging caused germination to drop an average of 13 percentage points. Earlier studies have shown that if longleaf seeds are dewinged carefully, germination is not reduced (Barnett 1969, Belcher and King 1968). Three

possible causes of dewinging damage are lack of seed drying, inappropriate dewinging equipment, and large size seeds.

Table 2. Longleaf pine cone and seed exposures during processing and resulting seed germination.

Variables	Collection date 1		Collection date 2		Avg.
	Plant A	Plant B	Plant A	Plant B	
			Cone and seed exposure		
Days of cone storage	44	42	28	20	
Kilning--total hours	96	92	119	94	
Kilning--hours >86°F	66	80	82	80	
Seed drying--total hours	117	21	116	17	
Seed drying--hrs. >86°F	60	19	58	17	
			Seed germination		
After kilning	59	52	76	81	67
After dewinging	46	46	59	65	54
After seed drying	54	47	64	67	58
Average	53	48	66	71	60

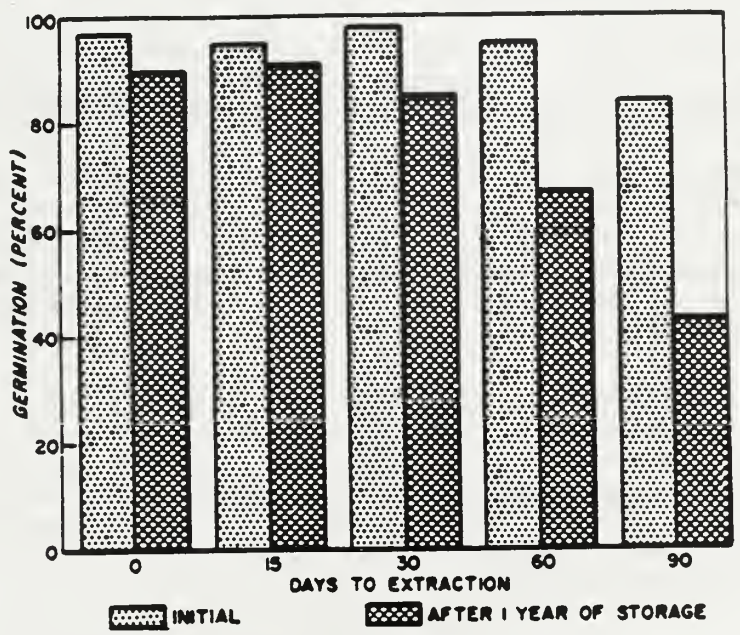

Figure 2. Effect of delayed extraction on longleaf seed germination initially and after one year of seed storage (from McLemore 1961).

First, processors may dewing seeds before drying for storage. Although this seems to be a logical approach, earlier studies have shown less damage to seeds dried before dewinging (Barnett and McLemore 1970). Drying results in more brittle wings that are quickly and easily reduced to stubs.

Second, equipment designed for optimal dewinging of loblolly (P. taeda L.) and slash (P. elliottii Engelm.) seeds may cause damage to the more sensitive longleaf seeds (Barnett and Pesacreta 1993). Many tests have shown that the harshness and length of dewinging must be minimized. Clearly, equipment must be modified to prevent injury to longleaf pine seeds.

Third, fertilization and other cultural practices in the orchard usually produce relatively large seeds (McLemore 1975b). Larger seeds are more likely to be damaged during processing because the portion of the seedcoat of total seed weight is less than in smaller seeds within the species. Sizing of longleaf seeds may be desirable to improve uniformity of germination in the nursery. A gravity table can be used to size seeds and remove empty or partially developed seeds that have lower viability.

Longleaf pine seeds are known to be relatively sensitive to high temperatures (Barnett 1979a, Rietz 1941), the length of exposure to high drying temperatures may reduce seed quality. In 1996, additional tests were conducted to evaluate a range of seed drying conditions on germination. Seeds used were from Stuart Orchard cones that were collected when fully mature and stored for 3 weeks prior to extraction. All seed drying treatments reduced and slowed germination, but maximum reduction was only 6 percentage points (table 3). There were no differences in germination among any of the drying treatments. Hence, seed drying does not seem to be a major cause of germination loss.

Table 3. Effects of seed drying conditions on germination of longleaf pine seeds.

Treatments	Length of exposure	Max. temp.	Avg. temp.	Avg. Rel. Humidity	Germ.	Germ. value[1]
	-hrs.-	---°F.----		---percent---		
Control (no drying)	--	--	--	--	96	50.5
Kiln @ 110°F.	30	110	108	23	91	37.5
Kiln @ 95°F.	51	100	96	34	91	39.9
Oven @ 120°F.	9	119	113	16	93	39.1
Ambient lab. temp.	339	77	71	56	90	37.1

[1]Germination values represent the speed as well as completeness of germination (Czabator 1962).

STORING SEEDS

The critical factors affecting storage are seed moisture content and storage temperatures. Results of long-term storage tests with longleaf pine seeds show that seeds must be dried to moisture contents below 10 percent and sealed in airtight containers (Barnett and Jones 1993). Tests have shown that longleaf pine seeds can be satisfactorily stored for 3 years or less at temperatures slightly above freezing (34°F) (Barnett 1969, Jones 1966). For longer periods, storage should be at subfreezing temperatures, preferably near 0°F (figure 3). Longleaf pine seeds have retained their viability for 20 years when held at 0°F temperatures (Barnett and Jones 1993). Seed quality can be maintained for periods to meet all practical needs. In fact, because damaged or less vigorous seeds are best preserved by lowering storage temperatures (Kamra 1967), the lower temperatures are recommended as a routine practice.

TREATING SEEDS

Although early research had suggested that some seedlots might benefit from short periods of stratification (USDA Forest Service 1948, Wakeley 1954), caution was urged because longleaf pine seeds frequently begin to germinate during stratification. As knowledge about how to properly collect, process, and store longleaf seeds increased, most researchers and practitioners felt stratification was unnecessary. In recent years, however, renewed interest in stratification of longleaf pine seeds has occurred--a result of the desire to upgrade or improve performance of seedlots of poor quality. Karrfalt (1988) reported that stratification for 14 days improved both speed and total germination in almost all 54 longleaf pine seedlots tested; most of which had relatively low viability.

Others provide data showing that stratification, while hastening germination by about 2 days, reduces total germination by about 10 percentage points (Barnett and Jones 1993). The disparity in these results may relate to the method of imbibition needed for stratification or test procedures. Operationally, seeds are stratified by soaking them overnight in water, draining the water, placing the seeds in polyethylene bags, and holding the bags under refrigeration for an appropriate period. Karrfalt (1988) placed the seeds in germination dishes and imbibied them with the germination medium. Barnett and Jones (1993) soaked the seeds in water alone for 16 hours which reduced germination by 10 percentage points.

Longleaf seedcoats are hosts to significant populations of pathogenic fungi (Barnett and Pesacreta 1993, Pawuk 1978). Germination of less vigorous seeds may be improved by treating with a sterilant, such as hydrogen peroxide (Barnett 1976b), or applying a fungicidal drench with benomyl (Barnett and Pesacreta 1993). Both treatments are used in southern forest nurseries.

CONCLUSIONS

Longleaf pine seeds are sensitive to injury during collection, processing, storage, and treatment. Because longleaf pine seeds are large, have relatively less dense coats, and are difficult to dewing, the techniques

used for processing other southern pines are inadequate. However, when properly handled, high quality longleaf pine seeds can be produced.

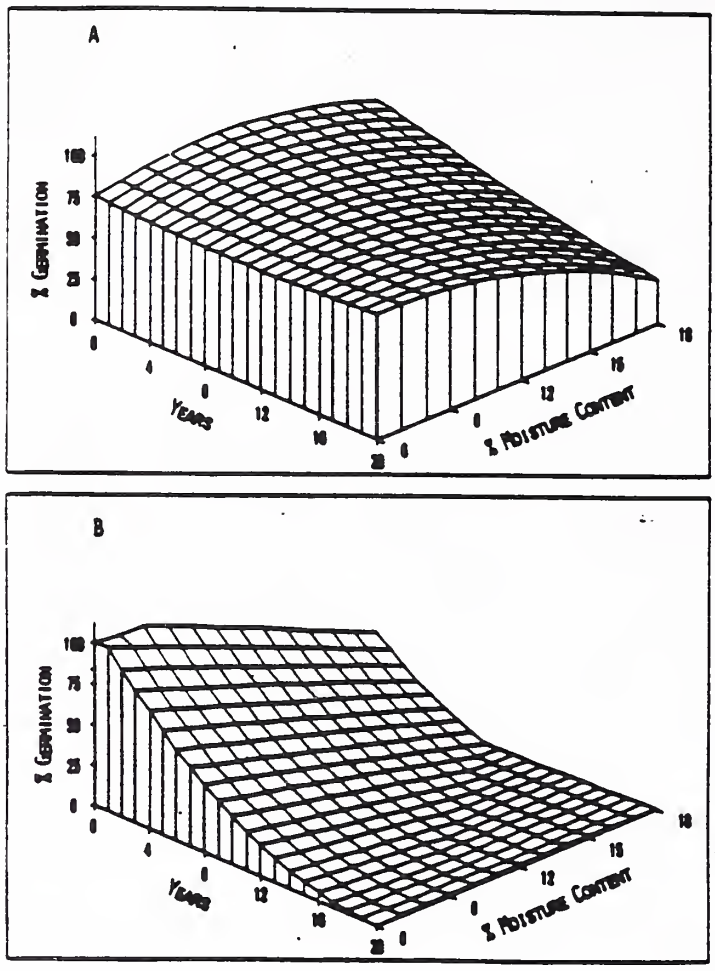

Figure 3. Germination of longleaf pine seeds as influenced by moisture content and years of storage at 0° (top) and 34°F (bottom).

RECOMMENDATIONS

The following recommendations include factors essential to maintaining high quality longleaf pine seeds:

1. Collect longleaf pine cones when fully mature (19 of 20 cones have a specific gravity of 0.89 or less) and hold for 3 weeks before processing. Do not delay processing of cones beyond 4 to 5 weeks.
2. Maintain kiln temperatures between 95°F and 105°F. As soon as the cones open, remove seeds from the kiln. Dry seeds to moisture contents below 10 percent by placing in seed dryers on clear, dry days when the ambient relative humidity is low.

3. Use dewinging equipment designed for longleaf pine to ensure that the wings are reduced to stubs without injury to the seedcoats. Dewing the seeds only when the wings are dry and brittle.
4. Remove trash, wings, and empty seeds carefully in a cleaning mill, on a gravity table, or by flotation in n-pentane (Barnett 1971).
5. Store in sealed containers at moisture contents less than 10 percent and at subfreezing temperatures, preferably near 0°F.
6. Conduct germination tests when seeds are placed in storage and if storage is longer than 1 year, again before use. If stratification is considered, conduct paired germination tests (stratified and control lots). Tests should follow presowing treatments that duplicate operational procedures, i.e., water soaking as used in stratification.
7. Consider control of seed microorganisms if lots are of low quality. The use of sterilants or fungicide soaks will significantly reduce populations of microorganisms on the seedcoats and may improve seed performance, particularly under nursery conditions.

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WOOD DENSITY ASSESSMENT OF DIVERSE FAMILIES OF LOBLOLLY PINE USING X-RAY DENSITOMETRY

Paul Belonger[1], Steven. E. McKeand[2], and J.B. Jett[2]

Abstract:--A direct scanning x-ray densitometer was used to measure juvenile wood density characteristics for two 10-year-old plantings of a genetics trial that included 52 open-pollinated families from four provenances of loblolly pine. Both volume production and wood density differed greatly for the two sites. Genetic parameters were estimated for basal area weighted whole-core density, earlywood and latewood density, and percent latewood. All traits were under high genetic control and exhibited strong family differences. Family mean whole-core densities ranged from 441 kg/m^3 to 507 kg/m^3. Site effects had a major affect on all these traits measured accounting for 42% to 73% of total variation, but significant genotype x environment interaction was not detected.

Linear regression models (density = ring number) were fit for individual tree annual earlywood, latewood, and ring densities to determine the rate of change in the traits over the first 10 years. Provenance differences were marginally significant ($p<0.10$) for the slope of the regression line for earlywood density and ring density. The individual tree narrow sense heritabilities for slope ranged from 0.05 to 0.15.

Keywords: Densitometry, *Pinus taeda*, Regression, Heritability, Correlation

INTRODUCTION

Gains in growth rate and quality traits generated by cooperative tree improvement programs have resulted in large increases in plantation productivity and the value of harvested trees (Talbert, et al. 1985). In addition, Jett and Talbert (1982) estimated that cooperators in the North Carolina State University-Industry Cooperative Tree Improvement Program that selected first generation orchard clones on the basis of average or above average wood density achieved a 2.6% gain in the wood density of the resultant improved plantation trees.

Several authors including Jett and Talbert (1982), Zobel and van Buijtenen (1989), and Zobel and Jett (1995) have reported that benefits beyond growth and form improvement can be realized by including wood density in selection strategies for advanced generations. Only a few programs (e.g., Anonymous 1995) have actually incorporated wood density into selection indices. To do so will require additional information describing the genetic and environmental variation of wood density. This paper describes the variation of wood density and related traits for 52 families representing four provenances of loblolly pine.

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MATERIALS AND METHODS

Plant Material

Wood samples for this study were taken from two of the remaining six installations of a loblolly pine provenance–progeny study jointly established by members of the North Carolina State University-Industry Cooperative Tree Improvement Program and the University of Florida Cooperative Forest Genetics Research Program (Table 1). The original objective of the plantings was to determine if two of the southern sources of loblolly pine (Marion County, Florida and Gulf Hammock, Florida) could be incorporated into a single coastal loblolly pine breeding population spanning from South Carolina to Mississippi (Anonymous 1988; Anonymous 1994).

The trials were planted in 1982 and included representative open-pollinated families from four geographic provenances (Figure 1) of loblolly pine: 1) Georgia–South Carolina Atlantic Coastal Plain (ACP), 2) Marion County (MC), Florida, 3) Gulf Hammock (GH) area of Florida located in Levy and Dixie Counties, and 4) Lower Gulf (LG) region of Mississippi and Alabama. These provenances are distinguished largely by geographic separation and the soil types (Vasquez 1993) on which they developed. The seeds for these plantings were produced in provenance-specific first generation open-pollinated seed orchards.

Table 1. Summary of location information.

Test Location	FL	AL
Latitude (N)	$31^{0}38'$	$31^{0}54'$
Longitude (W)	$81^{0}37'$	$86^{0}45'$
Elevation (ft.)	23	449
Mean Annual Rainfall (in.)	54	56
Mean Annual Temp. (^{0}F)	68	68
Soil Series	Leon	Orangeburg
Soil Drainage Class	Very Poorly	Well
Mean Tree Height (ft.)	34	55
Mean Tree Diameter (in.)	5.4	7.1

Note: Tree growth values reflect age 10 years measurements.

The locations and replications used in the present study were selected based on family representation of the four provenances as affected by mortality and level of rust infection. Every effort was made to attain balance in the dataset. Analysis of 10-year growth data was used to identify three of the five replications at each location having the greatest environmental uniformity.

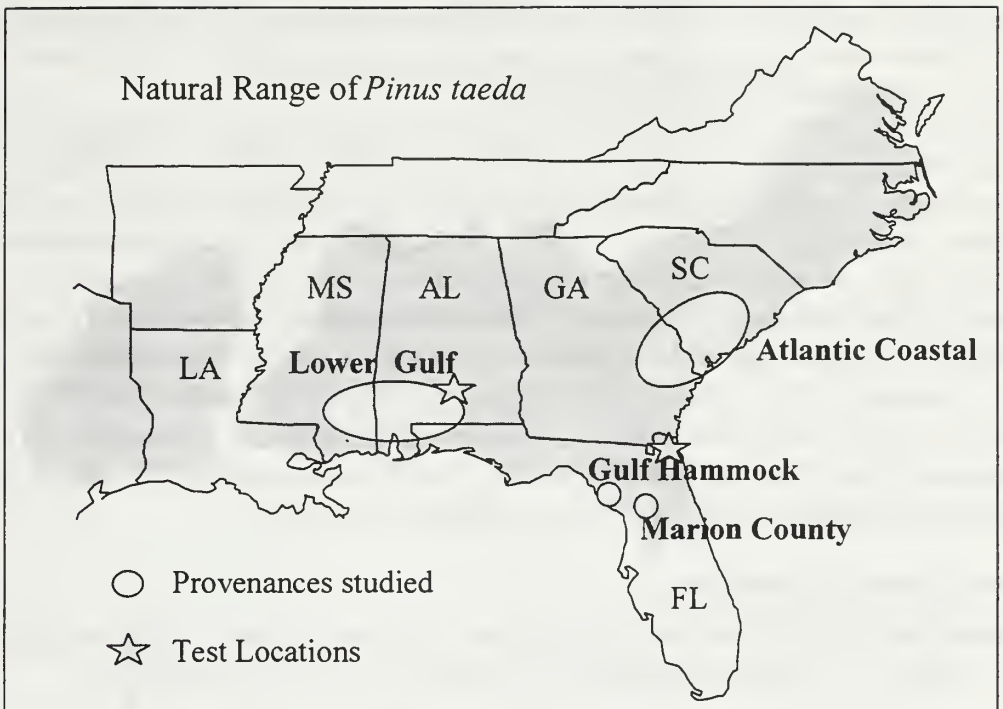

Figure 1. Origin of families and location of genetic tests

Sampling Procedure

Bark-to-bark 12mm increment cores were extracted from up to five dominant or codominat trees from each family in the three replications sampled from each location. Cores were extracted at breast height with minor allowances to avoid the confounding influence of compression wood caused by limbs and fusiform rust (*Cronartium quercuum* [Berk] Miyabe ex Shirai f. sp. *fusiforme*). Trees that were suppressed or had excessive stem rust were not sampled.

Each core was divided at the pith and ovendry weight - green volume unextracted specific gravity was determined (Zobel and van Buijtenen 1989) for one of the radial cores. Results from the analysis of these data (from four test sites) was reported by Belonger et al. (1996). The opposing half of the core was prepared for density data collection using the direct scanning x-ray densitometer housed in the tree improvement laboratory at North Carolina State University. Details of sample preparation methods and use of the densitometer can be found in Harding (1996).

Statistical Analysis

In general, the analyses were directed toward an evaluation of the variation available among the families. Since all families studied are well adapted to the Atlantic Coastal and Gulf Coastal Plains within the natural range of loblolly pine, they were viewed as a single

population in terms of deployment. However, the full model for the test design used in this investigation is shown below as it was used to estimate total variation in wood density traits and for provenance level analyses. The varcomp procedure of SAS (SAS Institute Inc. 1988) was used to estimate variance components.

$$Y_{ijklm} = \mu + L_i + B(L)_{ij} + P_l + PL_{li} + PB(L)_{lji} + F(P)_{kl} + LF(P)_{ikl} + F(P)B(L)_{ijk} + \varepsilon_{ijklm}$$

Where: Y_{ijklm} = individual observation for the kth half-sib family of the lth provenance in the jth block of the ith location; μ = fixed experimental mean; L_i = random effect of the ith location; $B(L)_{ij}$ = random effect of the jth block nested within the ith location; P_l = random effect of the lth provenance; PL_{li} = interaction effect of the lth provenance with the ith location; $PB(L)_{lbi}$ = interaction effect of the lth provenance with the jth block within the lth location; $F(P)_{kl}$ = random effect of the kth half-sib family of the lth provenance; $LF(P)_{ikl}$ = interaction effect of the ith location with the kth half-sib family of the lth provenance; $F(P)B(L)_{kpji}$ = interaction effect of the kth half-sib family of the lth provenance with in the jth block nested within the ith location; ε_{ijklm} = within plot residual error term.

Genetic Parameter Estimates

Individual tree heritabilities were calculated according to Falconer (1989) assuming the trees of a given family are related as half-siblings (Squillace 1974) using the formula:

$$h_i^2 = \frac{G}{P} = \frac{4\sigma^2_F}{\sigma^2_F + \sigma^2_{FL} + \sigma^2_{BF(L)} + \sigma^2_e}$$

Where: h_i^2 = individual tree heritability estimate; G = additive genetic variance; P = phenotypic variance among individuals; σ^2_F = family variance component; σ^2_{FL} = variation due to the interaction of families with locations; $\sigma^2_{BF(L)}$ = variation due to the interaction of families and blocks within locations; σ^2_e = within plot variance.

The genetic correlation between traits was estimated as:

$$r_{Ga,b} = \frac{COV_{Fa,b}}{\sqrt{\sigma^2_{Fa} * \sigma^2_{Fb}}}$$

Where: $COV_{Fa,b}$ = family covariance between traits a and b = $(\sigma^2_{Fa+b} - \sigma^2_{Fa} - \sigma^2_{Fb}) / 2$, and $\sigma^2_{Fa+b}, \sigma^2_{Fa}, \sigma^2_{Fb}$ = family variance for traits a+b, a, and b, respectively.

Standard errors of heritability and genetic correlation estimates were computed using methods of Becker (1992).

Regression Approach

Loblolly pine is known to have two distinct phases of wood production: juvenile and mature (Zobel and van Buijtenen 1989; Clark and Saucier 1991). The period of juvenility (Figure 2) normally lasts from the time wood is produced at the pith for about 10 growing seasons. During that time the rate of increase in ring density and latewood density is very similar and nearly linear. Earlywood density shows very little change over time and has a slope near zero.

Simple linear regression was used to characterize the density profile for earlywood, latewood, and average ring density. Regressions were run on individual tree data following the model:

$$\text{Density (Y)} = b_0 + b_1(\text{ring number from pith})$$

Variation associated with the first year of growth was eliminated by removing the first growth ring from the analysis as is common in such studies. The first ring from the pith is generally omitted due to the occasional presence of knots and resin pockets and because the pith of the tree is not always included in the core.

The regression coefficients (intercept and slope) were analysis using the general linear model and varcomp procedures of SAS (SAS Institute Inc. 1988) to evaluate effect differences and to estimate variance components.

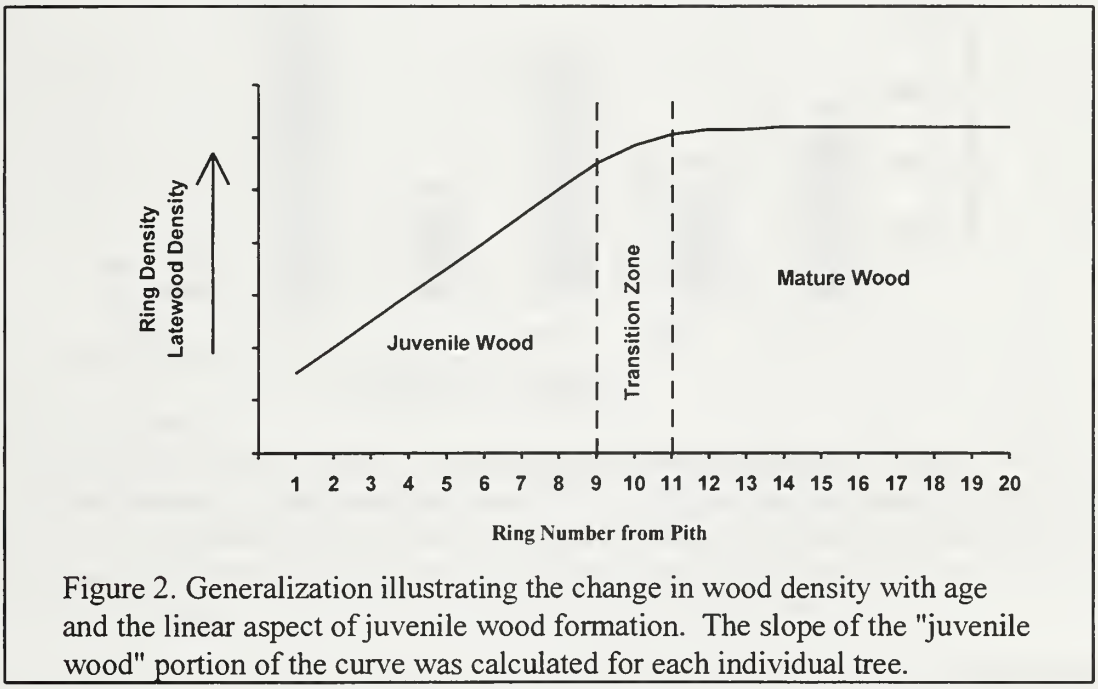

Figure 2. Generalization illustrating the change in wood density with age and the linear aspect of juvenile wood formation. The slope of the "juvenile wood" portion of the curve was calculated for each individual tree.

RESULTS AND DISCUSSION

Contribution to Total Variation

Whole-core Densities

Ring density in this study was most affected by environmental influences, namely test location, accounting for 73% of the total variation. This agrees with findings by Belonger et al. (1996) following analysis of the gravimetric data from four of the six provenance/progeny trials. Similar results have been reported by numerous authors (Zobel and van Buijtenen 1989). Site effects accounted for more than 50% of total variation for the four major density related traits except latewood density (42.1%). Location means for all density related traits

studied are shown in Figure 3. Latewood percentage showed the largest difference (29.4%) comparing the two sites.

Provenance level contributions to total variation were near equal for the four major traits and each was less than 2%. No provenance contribution was detected for earlywood density. The greatest contribution from family effects were found for earlywood and latewood densities, 4.0% and 4.1% respectively. There was no evidence of GxE as provenances and families were very stable across the two sites that differed greatly in productivity (Florida = 6.1 ft^3/tree; Alabama = 2.5 ft^3/tree).

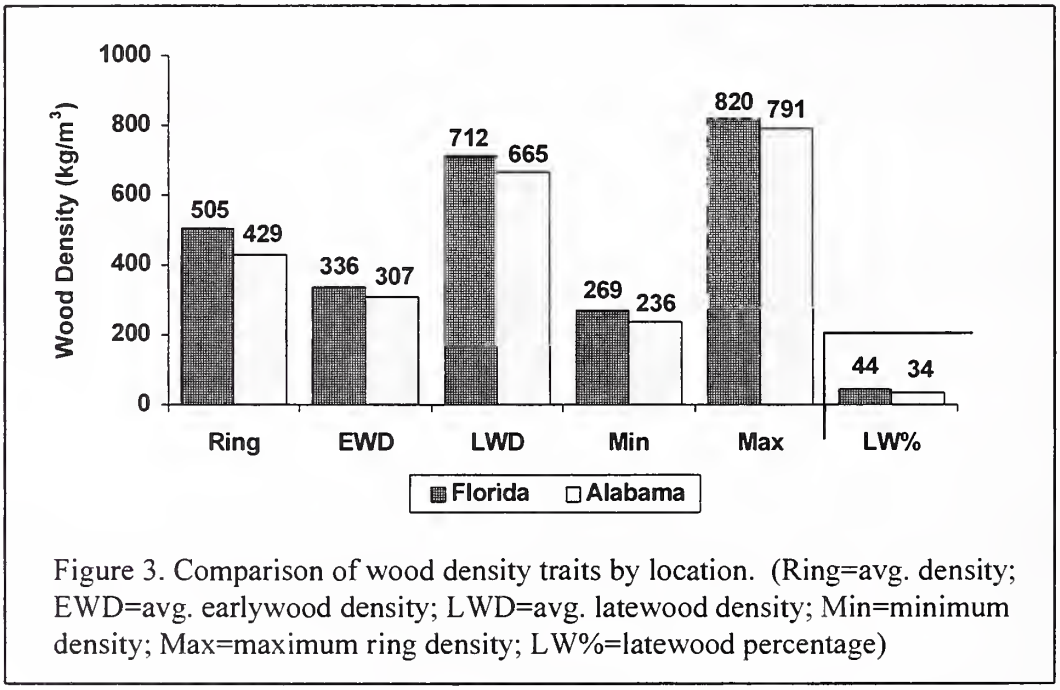

Figure 3. Comparison of wood density traits by location. (Ring=avg. density; EWD=avg. earlywood density; LWD=avg. latewood density; Min=minimum density; Max=maximum ring density; LW%=latewood percentage)

Regression Analysis

Contributions to the variation in the intercept and slope coefficients for the regression of wood density on ring number contrasted dramatically with each other (Table 2). The range in values for the contribution to the intercept approximated those for whole core densities except that test location had a smaller impact and the tree to tree variation was higher. The location and tree to tree variation values stand out when comparing the values for the intercept to those for the slope of the regression line. It's reasonable for the contributions of the intercept terms to approximate those for the whole core densities since the intercept could be used as a surrogate for the mean whole core values.

The lack of contribution of test location to variation in the slope of the regression line suggests that the environment in which trees are grown has minimal impact on year to year increase in ring and latewood densities during the juvenile period of wood formation. This is

true if the assumption regarding the linear relationship of wood density to ring number for the growth period studied is correct.

Table 2. Percent contribution of environmental and genetic effects to total variation in the ntercept and slope terms for the regression of wood density on ring number from pith. Loc=location; Rep=replication; Prov=provenance; Fam=family.)

	Intercept			Slope		
Provenance	Ring	Earlywood	Latewood	Ring	Earlywood	Latewood
Loc	49.0	34.4	28.2	0.0	0.0	0.0
Rep(Loc)	1.2	5.2	0.6	9.8	4.4	0.0
Prov	3.5	0.5	0.0	1.7	1.9	0.3
Prov*Loc	0.0	0.0	2.1	0.0	0.0	0.0
Prov*Rep(Loc)	2.1	0.8	4.3	3.8	2.0	4.6
Fam(Prov)	1.2	1.3	3.4	0.6	1.7	0.7
Loc*Fam(Prov)	0.0	0.0	0.0	0.5	1.2	0.0
Rep(Loc) *Fam(Prov)	3.2	6.9	1.9	3.0	0.0	4.6
Within plot	39.7	50.9	59.6	80.6	88.8	89.7

Genetic Effects and Parameter Estimates

Whole-core Densities

Provenance means for wood density and growth traits are shown in Table 3. These results agree with Jett et al. (1991) and Byram and Lowe (1988) who reported that faster growing southern sources of loblolly pine have lower wood densities when grown with more northern sources on the same site. Belonger et al. (1996) reported a slight negative genetic correlation between wood density and growth for the same families studied here.

As reported elsewhere the relative proportion of earlywood to latewood (latewood percentage) is the primary determinant of overall ring density; especially after age five (Megraw 1985). In this study the two provenances with higher ring densities had latewood percentages 6.5% higher than the two less dense Florida sources. This is explained in part by the association between the cessation of height growth and the onset of latewood production at the provenance level (Jayawickrama et al. 1997). Because height growth of the more southern sources of loblolly pine extends longer into the fall, less latewood is produced prior to dormancy. The estimated genetic correlation between ring density and latewood percentage in the present study was 0.977 (0.008) which further illustrates the importance of this trait.

Individual tree narrow sense heritabilities were calculated for ring, earlywood, and latewood densities and latewood percentage and were 0.39, 0.32, 0.40, and 0.34, respectively. The

heritabilities may be inflated by provenance effects, but reflect the amount of variation available to tree breeders within southern sources of loblolly pine.

Table 3. Provenance means for whole core density and growth traits at age 12 years. Sources joined by the same letter are not significantly different at p=0.05.

	Wood Density (kg/m^3)					
Provenance	Ring	Earlywood	Latewood	Latewood %	Height (ft.)	DBH (in.)
Atlantic Coastal	479 a	321 a	693 a	41 a	45.3 a	6.6 a
Lower Gulf	480 a	321 a	697 a	40 a	42.7 b	6.3 a
Gulf Hammock	465 b	325 a	679 b	38 b	46.9 a	6.9 a
Marion County	463 b	320 a	685 ab	38 b	45.9 a	6.7 a

Regression Analysis

The linear regression of wood density on ring number produced a typical relationship among the three density traits (Figure 4). Families had significantly different intercept and slope terms as expected based on the contributions to their variation (Table 2).

Differences among provenances for the intercept term for ring density (p=0.022) were equivalent to those for whole core ring density (p=0.012). However, the level of significance for provenance effects on the intercept term for earlywood and latewood was reversed relative to the whole core traits. The intercept for latewood was not different among the four provenances (p=0.655), but source effects were marginally significant (p=0.064) for earlywood.

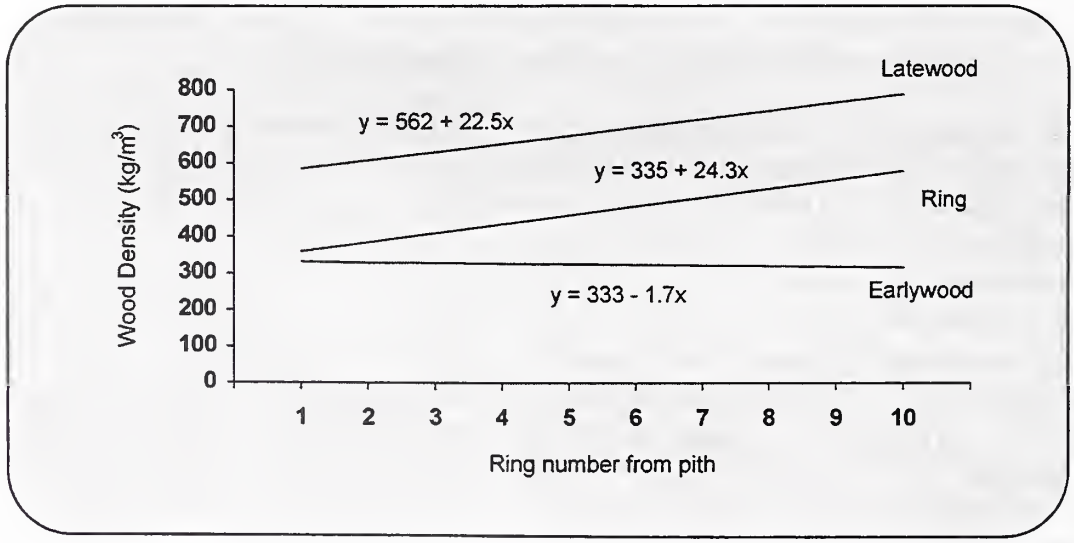

Figure 4. Linear change in wood density over time. The equations summarize the average effect of age from pith on wood density on an individual tree basis. (y=wood density, x=ring number.)

Family differences for the intercept term within the provenances were greatest for ring density and latewood density ($p<0.01$) and marginal for earlywood ($p=0.79$). No important GxE was detected at the provenance or family level.

The analysis of the slope term showed marginal provenance differences for ring density ($p=0.089$) and earlywood density ($p=0.064$), but no significant family effects. The Lower Gulf provenance had a higher average intercept and a smaller average slope compared to the other three provenances. As was the case throughout all analyses reported here no significant GxE was detected at the provenance or family level. The individual tree narrow sense heritabilities for the slope term were low for ring, earlywood, and latewood density were 0.10, 0.15, and 0.05, respectively.

The slopes of the regression lines indicate the rate of increase in wood density over time during the period of juvenile wood formation. In fact, analyses were not performed to clearly confirm this assumption and some families may have entered the transition zone while others may have begun to produce mature wood (Figure 2). Additional analyses (Belonger 1997) will investigate this relationship more closely.

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DIALLEL CROSS IN *Pinus cembra*: II THE NURSERY TEST AT AGE 6

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Abstract. A 10 x 10 full diallel cross was made in a native population of *Pinus cembra* L. from high elevation, to provide information about genetic variation and inheritance of important breeding traits, for the species. In October 1991, seeds were sown in individual polyethylene pots, in spruce humus. The families, including selfed and open pollinated parents, were arranged in a randomized complete block design with 4 replication and 12 seedlings per plot. Six traits were measured, prior to field planting, when the progenies were 6 years old: total height (H.6), height increment in the 6th year (h.6), diameter at root collar (DRC), number of buds around the leader bud (NBAL), total number of branches (TNB) and lamma shoots formation (LS). In addition, the weights of 100 seeds (100 SW) of each family were measured prior to sowing and cotyledon number (CN) was counted after seed germination. Computer analysis of balanced modified full diallel using Schaffer and Usanis' DIALL program produced the results presented below. The most important result was that significant ($p < 0.05$) and highly significant ($p < 0.01$; $p < 0.001$) differences occurred in the all 8 traits for g.c.a., s.c.a., maternal and reciprocal effects. This suggest that the traits were controlled by nuclear (additive and non-additive) and extra nuclear genes, and by nuclear x extra nuclear gene interactions. Additive and non-additive genetic variances accounted for 25% and 27% for H.6 and 25% and 17% for h.6 of the phenotypic variance, respectively, indicating that both variances were important for height growth in this population. There were found parents with significant g.c.a. effects for growth and other traits. Narrow - sense heritability estimates varied between 0.081 to 0.477 for CN and LS, respectively, while the H.6 accounted for 0.292. By selecting the best 10 to 40 families, a genetic gain in H.6, between 6.0% and 3.1% could be achieved. In conclusion, the improvement of the growth by using both additive and non - additive gene effects should be possible.

Key words: *Pinus cembra*, diallel cross, additive variance, combining ability, genetic correlation, heritability.

INTRODUCTION

The natural distribution area of the stone pine (*Pinus cembra* L.) is restricted to the high elevations of the Alps and Carpathians (Holzer, 1963; Critchfield and Little, 1963). In the Alps, the species ranges between 1200 and 2500 m elevation (Contini and Lavarelo, 1982) but the main zone is between 1500 and 2000 m (Holzer, 1975). In Romania, stone pine ranges between 1350 and 1880 m elevation in the northern Carpathians (Gubesh, 1971) and between 1350 and 1986 m in the southern Carpathians (Beldie, 1941; Tataranu and Costea, 1952; Oarcea, 1966).

The stone pine is important for : (1) reforestation of the subalpine zone to raise the timber line, to its initial limit, where it plays a leading part in watersheds, for stabilizing avalanche areas

and for reducing the effects of the flash floods (Holzer, 1972a, 1975); (2) spruce-larch-cembra mixed stands creation at high elevation in order to increase their windbreak resistance (Blada, 1996); (3) its dense - brown - reddish wood useful for handicrafts (Contini and Lavarelo, 1982); (4) its high resistance to blister-rust caused by *Cronartium ribicola* Fisch. ex Rabenh. (Bingham,1972; Holzer,1975; Hoff et al., 1980; Blada, 1982; 1987; 1990a; 1994); (5) landscaping purposes due to its conic - oval shape when grown as single tree (Blada, 1996);

Insofar, as can be determined, not too many breeding work with stone pine have been reported. Until now, the main experiments have concentrated on cone and seed studies (Rohmeder and Rohmeder,1955, Nather 1958, Holzer 1972b; Blada and Popescu, 1992), provenances testing (Blada,1997), half-sib families testing (Holzer, 1975; Blada, 1996), full-sib families testing (Blada, 1995) and interspecific hybridization (Blada, 1987; 1994).

A genetic improvement program with stone pine have been started in Romania (Blada, 1990 b, 1995) that has the following objectives : (1) phenotypic selection of parents in natural populations; (2) testing provenance and half-sib families; (3) intra and interspecific crosses; (4) full-sib families testing, estimates of genetic parameters and selection of the best combiners and families; (5) seed orchard establishment with the best combiners for both mass seed production and base population for advanced - generation breeding. Four papers, as part of this program, have been published until now (Blada and Popescu, 1992; Blada, 1994, 1995, 1997), and one is presented now. The objective of this paper was to provide information about genetic variation and inheritance of important breeding traits useful in the breeding of the stone pine.

MATERIALS AND METHODS

Initial material and mating design

The 10 parents trees used in the crossing scheme were randomly chosen from Gemenele old natural population located in the Retezat Mountains from Southern Carpathians, at about 1800 m elevation. Actually, reproductive fertility was taken into consideration in parent selection in order to obtain the necessary number of flowers for pollination. The flowers were isolated in paper bags prior to local pollen dissemination. Fresh pollen was used for all crossings. A full - diallel mating design according to Griffing's (1956) Method 1 was used, during July 1989.

Progeny test and experimental design

One hundred seedlots from controlled crossing were collected in October 1990. Before sowing, the filled seeds were separated from empty seeds by immersing each seedlot in 90° alcohol. This procedure was good because only the full seeds were sown; but, from statistical standpoint, it may not be correct because it produced a decrease of environmental error variance and an increase of other components of phenotypic variance, such as SCA and reciprocal variances.

Seeds was sown in November 1989 in individual polyethylene pots (22cm x 18cm) in a potting spruce humus. Based on results of previous local experiments (Blada, 1996), by sowing the stone pine seed during the autumn, the seed develop its embryo and germinate in the next spring eliminating a complicated, costly and risky 180 days stratification period as Kriebel (1973) recommended. But, if sowing was done during autumn, control measures for seed predation by mice

in the nursery beds was compulsory.

After sowing, the seeded pots were placed in nursery beds and arranged in a randomized complete block design. Each of 100 families was represented by a 12 seedling plot in each of 4 blocks. The seedlings were kept in pots throughout the 6 years testing period.

Measurements

Six traits were measured, prior to field planting, when the progenies were 6 years old. In addition, the weights of 100 seeds of each family were measured prior to sowing and cotyledon number was counted after seed germination (Table 1). Lamma shoots was measured using 5 indices: 1 = no lamma shoots; 2 = only a small number of new needles present; 3 = slight flushing on leader and secondary branches; 4 = leader and / or branches with 1 - 2 cm growth; 5 = leader and / or branches with more than 2 cm of growth. The other traits listed in table 1, do not require additional explanation. The plot means comprised the basic data for statistical analysis.

Statistical analysis

Although initially a full diallel mating design was used, the analysis was performed according to the modified full diallel mating design known as Griffing's (1956) Method 3, where the parents (selfed) were excluded; such analysis leads to unbiased estimates. However, the parents were included in the material grown in the experiment so that comparisons of hybrids with their parents could be made in other type of analyses.

The mathematical model for analysis was a combination of Hayman (1954a) and Griffing's (1956) models, such as:

$$x_{kij} = u + b_k + g_i + g_j + s_{ij} + m_i - m_j + r_{ij} + e_{kij} \qquad (1)$$

where: x_{kij} = the mean performance in k-th block of the i-th parent mated to the j-th parent; u = the general mean; b_k = the effect of the k-th block; g_i and g_j=the general combining ability effects for the i-th and j-th parents, respectively; s_{ij} = the specific combining ability effect for the cross between the i-th and j-th parents so that $s_{ij} = s_{ji}$; m_i and m_j = the maternal effects of the i-th and j-th parents; r_{ij} = the difference caused by the direction of the cross between i-th and j-th parents, such that $r_{ij} = -r_{ji}$; e_{kij} = the random error.

Plot means of the eight measured traits were analysed using the least - squares method by means of the computer DIALL program prepared by Schaffer and Usanis (1969). The analysis of balanced modified full diallel according to Griffing's (1956) Method 3, was based upon the random model assuming that the parents were random samples from a random mating population. This assumption make possible estimates of the additive and non-additive genetic variance of the parent population.

The model of analysis of variance, expected mean squares and formulas for estimating the variance components were listed in table 2.

Standard errors (SE) of variance components were computed with the formula given by Anderson and Bancroft (1952):

$$SE(\sigma^2_j) = \sqrt{\sum_i \frac{2a_i^2 (MS_i)^2}{df_i + 2}} \qquad (2)$$

where: a_i are the coefficients of the inverse of the matrix of expected mean squares used to estimate the j-th variance component.

The component of variance σ^2_{GCA} was used to estimate the variance in general combining ability among all of the parents in this experiment and is used as an estimator of 1 / 4 σ^2_A. It is assumed that all epistatic components of genetic variance were insignificantly small. The component σ^2_{SCA} , the estimated variance in specific combining ability, is an estimator of 1/4 σ^2_D.(with the same assumptions). Therefore, an estimator of the additive genetic variance is $4\sigma^2_{GCA}$ and an estimate of the dominance genetic variance is $4\sigma^2_{SCA}$.

The narrow-sense heritability estimates at half-sib family level (h^2) was calculated using the formulas given by Kriebel, et al. (1972), adapted to this case, as follows:

$$h^2 = \frac{\sigma^2_{GCA}}{\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{Mat} + \sigma^2_{Rec} + \sigma^2_p/k + \sigma^2_w/kn} \qquad (3)$$

where: σ^2_p = plot error = σ^2_e - σ^2_w/n ; σ^2_w = within plot error variance ; n = number of seedlings per plot; k = number of replications; the other symbols are as defined in table 2.

The general combining ability (g_i) effects were calculated by the computer according to the DIALL program (Schaffer and Usanis, 1969).

Genetic correlations (r_g) were directly calculated by the computer, according to the following formula (Falconer,1960):

$$r_g = \frac{cov_{GCA(xy)}}{\sqrt{\sigma^2_{GCA(x)} \sigma^2_{GCA(y)}}} \qquad (4)$$

where: $cov_{GCA(xy)}$ = additive covariance component between the traits x and y; $\sigma^2_{GCA(X)}$ and $\sigma^2_{GCA(Y)}$ = the variances due to GCA for traits x and y, respectively.

The genetic gain (ΔG) was calculated by Falconer's (1960) formula :

$$\Delta G = i\, h^2\, \sigma_P \qquad (5)$$

where i = intensity of selection taken from Becker (1984) and σ_P = phenotypic standard deviation.

RESULTS AND DISCUSSIONS

Genetic variation

The most prominent feature of this experiment was that significant ($p < 0.05$) and highly significant ($p < 0.01$; $p < 0.001$) differences occurred in all traits for g.c.a. , s.c.a. and reciprocal effects. Maternal effects were statistically significant ($p < 0.05$) for H.6, DRC and TNB and highly significant ($p < 0.01$; $p < 0.001$) for 100 SW and CN (Table 3). This suggest that all traits, including growth ones, were controlled by nuclear (additive and non-additive) genes and by nuclear x extra nuclear gene interactions, whilst the extra nuclear genes were involved only in five traits.

Table 4 presents the best and the poorest six full-sib families indicating large genetic variation among family means for several traits. Thus, the H.6 mean of the poorest 6 families averaged 19.7 cm whilst the best 6 families averaged 28.2 cm, i.e. 43% taller. The differences between the two groups were even larger for some other traits, such as : 96% for DRC, 46% for TNB and 94% for LS (see D_1 in table 4) In the same context, the mean top 6 families surpassed the test mean in 100 SW, CN, H.6, h.6, DRC, NBAL, TNB and LS by 22%, 5%, 18%,18%, 77%, 17%, 19% and 37%, respectively (see D_2 in table 4).

Low to high genetic variation coefficients were found among full-sib families (Table 7). The value of this coefficients varied between 3% for CN and 19% for LS, while the H.6 one accounted for 12%.

Large genetic variation was found not only among cross-pollinated families but among self-pollinated ones, as well. Two-way variance analysis of the 10 selfed parent families showed that significant ($p < 0.05$) and highly significant ($p < 0.01$; $p < 0.001$) differences were found among them for all tested traits (Table 5). This result demonstrated that the variation within S_1 population in still very high.

Surprisingly, higher genetic coefficients of variation (GCV) were calculated within self- than within cross-pollinated population, i.e., the GCV values of the self- and cross-pollinated families ranged between 10% and 37% (Table 5) and between 3% and 19% (Table 7) respectively. Self-pollinated families (SP) have strongly contrasted with those from cross-pollinated (CP) ones, in 7 out 8 traits (Table 6). A comparison of overall means indicated that, CP trees performed 52% and 39% better in H.6 and DRC, respectively, than SP trees. However, CP mean was almost equal in CN and lower in LS than SP mean.

In conclusion, progeny testing has demonstrated a high within population genetic variation that could be exploit in a breeding program.

Variance components

Variance component estimates, standard errors and dominance ratios were listed in table 7. The diallel analysis indicated that GCA and SCA variance components were important sources of variation for all eight tested traits. Additive and non-additive genetic variances accounted for 25% and 27% for H.6, 25% and 17% for h.6 and 14% and 22% for DRC, respectively, of the phenotypic variance. Therefore, the magnitude of GCA variance relative to SCA variance for these traits suggested that additive gene effects may be almost as important as non-additive ones, in this young population indicating that progress under selection is possible to a considerable degree.

Dominance variances exceeded additive ones in five out of eight traits, i.e. in 100 SW, CN, H.6, DRC and TNB. For example, the ratios of non-additive to additive variances were 2.1 : 1.0 for 100 SW, 1.1 : 1.0 for H.6 and 1.6 : 1.0 for DRC , indicating a clear over dominance in these traits, while a partial dominance was noticed in h.6, NBAL and LS with the following ratios: 0.7 : 1.0 and 0.9 : 1.0, and 0.4 1.0, respectively.

The maternal component of variances significantly contributed to the phenotypic variances. The largest maternal component of this study was 10% of the phenotypic variance and it was associated with 100 SW, while the smallest one of 1% was associated with both NBAL and LS. The maternal variance of the H.6 and DRC accounted for 4% and 5%, respectively, of phenotypic variance. Thus, with one exception, the experimental material of six years old, supported evidence that maternal effects were moderately large.

The reciprocal variance components accounted for between 6% for LS and 31% for 100 SW of the phenotypic variance. High proportion of reciprocal variance was calculated in H.6 and DRC, i.e. 25% and 21%, respectively. Consequently, the contribution of reciprocal variance was large in growth traits, accounting for about the same percentage of the phenotypic variance as did GCA and SCA variances. Therefore, reciprocal, as well as maternal variances could be important in a breeding program for stone pine.

It must be stressed that GCA, SCA, maternal and reciprocal variance component estimates were associated with small standard errors at all but one cases, indicating their reliability.

The above mentioned results offered support for adopting full diallel mating design (though it is very costly) for estimation not only GCA and SCA but maternal and reciprocal variance components, as well.

It was evident that all variances were well represented in almost all traits, suggesting that a selective breeding program utilizing not only nuclear but maternal gene effects, as well, could be adopted.

General combining ability

General combining ability effects calculated for each parent tree were listed in table 8. Both positive and negative g.c.a. effects which significantly differed from the test mean were found for 7 out of 8 traits. Parent X had the largest positive g.c.a. effects for 100 SW, H.6, h.6 and DRC, whereas parent Z was the second highest for both H.6 and h.6. On the other hand, parents 3 and 45 had the largest negative values for H.6 and h.6. Consequently, parents X and Z should be selected for their high positive g.c.a. effects and breeding value for growth traits whereas parents 3 and 45 and some others have to be rejected because of their high negative g.c.a. effects for the same growth traits.

In conclusion, if two out of ten randomly selected parents exhibited significant positive g.c.a. effects for growth, then one may assume that 20% of trees within natural population, (where the parents have been growing), could be selected as good combiners.

Correlations

Both phenotypic and genetic correlations between traits were calculated (Table 9). There were found significant ($p < 0.05$) and highly significant ($p < 0.01$; $p < 0.001$) positive phenotypic correlations between: 100 SW and H.6, 100 SW and h.6; 100 SW and DRC; CN and DRC; H.6 and h.6, H.6 and DRC, H.6 and NBAL, H.6 and TNB; h.6 and DRC, h.6 and NBAL, h.6 and TNB; DRC and NBAL, DRC and TNB.

Substantial positive genetic correlations were found between: 100 SW and DRC; CN and DRC; H.6 and h.6; H.6 and DRC; h.6 and DRC.

According to indirect selection principle, the strong positive genetic correlations between H.6, h.6 and DRC imply genetic gain in any of these traits even if selection was practiced on only one. On the other hand, due to some positive correlations one could expect some negative economical results. For example, total number of branches, will increase with H.6, h.6, DRC and NBAL, and for this reason a conscious effort should be made to find fast growing trees with a small number of branches.

The previous results needed to be confirmed from long-term juvenile-adult correlations and correlations with additional characters of economic and ecologic value.

Heritability and genetic gain

Estimates narrow-sense heritability on a plot mean basis were fairly consistent for five traits, such as: 0.292 for H.6, 0.354 for h.6, 0.293 for NBAL, 0.267 for TNB and 0.477 for LS. However, the heritabilities were low for the other three traits (Table 7).

Table 10 showed that the highest genetic gain could be achieved in LS. But this genetic gain is doubtful because lamma shoots formation in species growing in temperate or at higher latitudes could frequently be positive correlated with frost susceptibility. By selecting the best 10, 20, 30 or 40 out of 90 full-sib tested families, a genetic gain in H.6 and DRC of 6%. 4.7%, 3.9%, 3.1% and 2.7%, 2.1%, 1.7%, 1.4%, respectively, could be expected. Such a gain could be economically important if the improved planting material will be used on a large area. These gains at age 6 may be good predictors of later results. However, later age correlations, wich are not available, will be more reliable for final gain estimation.

It should be noted that the parental selections were random with regard to vigor and all were selected in a single native population. In this situation, some parents were probably related and there was, therefore, a closer than half-sib average relationship among the progenies. For this reason, heritabilities and genetic gains probably were underestimated.

The results indicated that selection on the basis of family comparisons could be effective and might be economically acceptable if the loss in selection differential and testing time could be made small.

CONCLUSIONS

The results illustrated the existence of sufficient additive as well as non-additive genetic variance within the breeding population for growth traits to utilize in an improvement program.

Maternal and reciprocal variance could also be taken into consideration for stone pine improvement.

Parents with a good general and specific combining ability for growth traits to be used in a breeding program could be found within *P. cembra* natural population where the parents have been growing. Narrow - sense heritabilities of the traits were low to fairly large.

The existence of substantial positive genetic correlations between several traits, including growth ones, suggested that indirect selection could be applied.

A genetic gain in growth, and other traits, could be achieved by selecting and planting the best families and individuals from tested population.

This test supports the adoption of a full diallel mating design in *P. cembra* even if it requires more effort than a half diallel one; by this way is possible to detect the maternal and reciprocal effects.

An effort to improve the growth traits by exploiting g.c.a., s.c.a. and perhaps maternal effects seems to be rewarding.

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Figure 1 . Full diallel mating design according to GRIFFING's (1956) Method 1

Parents	2	3	45	50	205	206	209	X	Y	Z
2	X	x	x	x	x	x	x	x	x	x
3	x	X	x	x	x	x	x	x	x	x
45	x	x	X	x	x	x	x	x	x	x
50	x	x	x	X	x	x	x	x	x	x
205	x	x	x	x	X	x	x	x	x	x
206	x	x	x	x	x	X	x	x	x	x
209	x	x	x	x	x	x	X	x	x	x
X	x	x	x	x	x	x	x	X	x	x
Y	x	x	x	x	x	x	x	x	X	x
Z	x	x	x	x	x	x	x	x	x	X

Table 1. Measured traits

Traits	Units	Symbol
100 seed weight	g	100 SW
Cotyledon number	No	CN
Total height at age 6	cm	H.6
Height increment in the 6-th year	cm	h.6
Diameter at root collar	mm	DRC
Number of buds around the leader bud	No	NBAL
Total number of branches	No	TNB
Lamma shoots	Index 1-5	LS

Table 2. Analysis of variance of modified full diallel, random effects model, in a randomized block layout in one environment.

Source	Df	MS	E (MS)	F-test
Replications (R)	k-1	MS_R	$\sigma^2_e + p\,(p-1)\,\sigma^2_{Rep}$	
GCA	p-1	MS_{GCA}	$\sigma^2_e + 2k\,\sigma^2_{SCA} + 2k\,(p-2)\,\sigma^2_{GCA}$	
SCA	p(p-3)/2	MS_{SCA}	$\sigma^2_e + 2k\,\sigma^2_{SCA}$	
Maternal (Mat)	p-1	MS_{Mat}	$\sigma^2_e = 2k\,\sigma^2_{Rec} + 2kp\sigma^2_{Mat}$	
Reciprocal (Rec)	(p-1)(p-2)	MS_{Rec}	$\sigma^2_e + 2k\,\sigma^2_{Rec}$	
Error	(k-1)(p^2-p-1)	MS_E	σ^2_e	
Total	kp(p-1)			

$\sigma^2_e = MS_E$; $\sigma^2_{Rec} = (MS_{Rec} - MS_E)/2k$; $\sigma^2_{Mat} = (MS_{Mat} - MS_{Rec})/2kp$; $\sigma^2_{SCA} = (MS_{SCA} - MS_E)/2k$; $\sigma^2_{GCA} = (MS_{GCA} - MS_{SCA})/2k(p-2)$; $MS_{Rep} = (MS_{Rep} - MS_E)/p(p-1)$

Table 3. Analysis of variance of modified full diallel of *P. cembra* tested traits

Source of variation	Df	Mean squares for the traits...							
		100 SW	CN	H.6	h.6	DRC	NBAL	TNB	LS
Repl..	3	2.963	0.241	5.407	2.880	1.865	0.459	3.542	0.434
GCA	9	133.113●●●	1.391●	182.958●●●	43.882●●●	12.155●●●	4.75●●●	8.761●●●	8.886●●●
SCA	35	27.715●●●	0.479●	23.779●●●	4.407●●●	2.425●●●	0.609●●●	1.321●●●	0.641●●●
Mat	9	93.261●●●	2.250●●	49.702●	8.487	6.572●	0.723	3.018●	0.911
Rec	36	23.026●●●	0.661●●●	21.540●●●	4.187●●●	2.298●●●	0.627●●●	1.039●●●	0.446●●●
Plot error	266	0.410	0.267	1.885	0.996	0.421	0.140	0.349	0.247

Table 4. Means of the 6 best (upper part) and the 6 poorest (lower part) full-sib families

Rank	Traits							
	100 SW	CN	H.6	h.6	DRC	NBAL	TNB	LS
1	24.7	10.8	29.7	12.9	12.6	4.4	5.4	3.5
2	24.3	10.6	28.6	12.8	11.8	4.4	5.2	3.4
3	23.3	10.4	28.2	12.3	11.8	4.3	5.1	3.4
4	21.7	10.4	27.6	12.2	11.6	4.2	5.0	3.4
5	21.5	10.3	27.5	12.2	11.5	4.1	5.0	3.1
6	20.9	10.3	27.4	12.0	11.5	4.1	5.0	3.0
Sub-mean	22.7	10.5	28.2	12.4	18.8	4.2	5.1	3.3
40	15.7	9.7	21.1	9.1	9.8	3.1	3.8	1.8
41	15.5	9.6	20.5	9.0	9.7	3.1	3.7	1.8
42	15.4	9.6	19.9	9.7	9.7	3.1	3.6	1.7
43	15.3	9.5	19.6	8.3	9.6	2.9	3.5	1.7
44	13.7	9.5	18.8	8.0	9.6	2.8	3.3	1.5
45	13.3	9.3	18.6	7.7	9.3	2.8	3.0	1.4
Sub-mean	14.8	9.5	19.7	8.5	9.6	3.0	3.5	1.7
Test mean	18.6	10.0	23.9	10.5	10.6	3.6	4.3	2.4
D_1(%)	53	11	43	46	96	40	46	94
D_2(%)	22	5	18	18	77	17	19	37

D_1 and D_2 = differences (%) between the poorest 6 and the best 6 families and between test mean and the best 6 families, respectively

Table 5. Two-way ANOVA of self-pollinated families, variance components (σ^2), standard errors (SE) and genetic coefficients of variation (GCV)

Source of variation	DF	Mean squares for the traits...							
		100 SW	CN	H.6	h.6	DRC	NBAL	TNB	LS
Replications(R)	3	1.29	0.38	4.17	0.34	1.17	0.11	1.02	0.29
Selfed-families	9	25.09●●●	2.08●	20.26●●	6.84●●●	3.18●	0.66●●●	4.18●●●	3.64●●●
Error	27	0.62	0.75	5.62	1.22	1.02	0.13	0.13	0.32
Components									
$\sigma^2_F \pm$ SE		6.12 ± 2.68	0.33 ± 0.23	3.66 ± 2.19	1.40 ± 0.73	0.54 ± 0.35	0.13 ± 0.07	0.99 ± 0.45	0.83 ± 0.39
$\sigma^2_e \pm$ SE		0.62 ± 0.16	0.75 ±0.20	5.62 ± 1.48	1.22 ± 0.32	1.02 ± 0.27	0.13 ± 0.03	0.23 ± 0.06	0.32 ± 0.08
σ^2_p		6.74	1.08	9.28	2.62	0.26	0.26	1.22	1.15
GCV (%)		14	10	19	24	16	27	37	33

Table 6. Performance comparisons between control-cross-pollinated (CP) and control-self-pollinated (SP) progenies from the same parents

Parents	100 SW g		CN No.		H.6 cm		h.6 cm		DRC mm		NRAL No.		TNB No.		LS Index	
	CP	SP	CP	SP	CP	SP	CP	SP	CP	SP	CP	SP	CP	SP	CP	SP
2	19	17.0	9.8	9.7	23.7	15.1	10.5	6.4	10.5	7.1	3.8	2.3	4.4	2.6	2.4	3.5
3	18	16.4	9.9	9.8	22.0	13.6	9.8	5.7	10.6	6.6	3.5	1.4	3.9	1.5	2.2	2.7
45	19	17.8	10.1	9.9	21.3	12.8	9.1	4.7	10.3	7.6	3.4	2.0	4.3	2.9	2.4	3.4
50	20	22.2	9.8	8.2	23.9	15.6	10.7	6.2	10.5	7.0	3.1	1.3	4.2	3.0	2.8	3.9
205	19	20.3	10.2	11.1	24.2	14.4	10.2	5.0	11.1	8.3	3.8	2.2	4.4	3.6	2.8	4.4
206	18	14.8	10.0	9.9	24.9	15.5	11.2	6.8	10.5	7.2	3.6	1.6	4.0	2.5	2.2	2.7
209	17	16.9	9.9	9.8	23.4	18.0	10.1	7.1	10.4	8.6	3.7	2.6	4.9	5.2	2.7	3.7
x	21	17.3	10.1	10.1	26.2	20.3	11.7	9.2	11.5	9.4	3.5	2.2	4.8	3.9	2.2	2.0
y	20	21.4	9.9	10.1	23.8	17.2	10.5	7.6	10.4	7.7	3.6	1.9	4.0	2.3	1.8	1.5
z	18	15.7	9.9	10.4	25.7	14.6	11.0	6.9	10.5	6.8	3.9	1.9	4.1	2.8	2.7	4.2
Mean	19	18.0	10.0	9.9	23.9	15.7	10.5	6.6	10.6	7.6	3.6	1.9	4.3	3.0	2.4	3.2
SUP (%)	5		1		52		59		39		89		43		-25	

SUP = superiority above the self-pollinated progenies

Table 7. Variance components (precents in brackets), standard errors, dominance ratios, genetic variation coefficients, narrow-sense heritabilities on both family and individual seedling basis

Components	100 SW	CN	H.6	h.6	DRC	NBAL	TNB	LS
σ^2_{GCA}±SE	1.6514(18) ±0.8951	0.0143(4) ±0.0095	2.4941(25) ±1.2255	0.6185(25) ±0.2936	0.1525(14) ±0.0817	0.0649(20) ±0.0318	0.1166(17) ±0.0587	0.1292(28) ±0.0594
σ^2_{SCA}±SE	3.4211(37) ±0.80789	0.0265(7) ±0.0143	2.7433(27) ±0.6934	0.4274(17) ±0.1289	0.2511(22) 0.0708	0.0588(18) ±0.0178	0.1218(17) ±0.0387	0.0497(11) ±0.0189
σ^2_{Mat}±SE	0.8806(10) ±0.50316	0.0199(5) ±0.0122	0.3530(4) ±0.2729	0.0539(2) ±0.0470	0.0536(5) ±0.0358	0.0012(1) ±0.0043	0.0248(4) ±0.0164	0.0058(1) ±0.0050
σ^2_{Rec}±SE	2.8364(31) ±0.6625	0.0494(13) ±0.0192	2.4650(25) ±0.6201	0.4002(16) ±0.1209	0.2354(21) ±0.0663	0.0610(18) ±0.0181	0.0866(12) ±0.0301	0.0249(6) ±0.0131
$\Sigma\sigma^2_G$	8.7895	0.1102	8.0554	1.5000	0.6924	0.1859	0.3498	0.2093
σ^2_e ± SE	0.4098(4) ±0.0354	0.2669(71) ±0.0231	1.8849(19) ±0.1628	0.9960(40) ±0.0860	0.4213(38) ±0.0364	0.1404(43) ±0.0121	0.3486(50) ±0.0301	0.2472(54) ±0.0214
σ^2_p	8.8919	0.1769	8.5266	1.7490	0.7978	0.22109	0.4369	0.2711
σ_p	2.9819	0.4206	2.9200	1.3225	0.8932	0.4701	0.6610	0.5206
$\sigma^2_{SCA}/\sigma^2_{GCA}$	2.0717	1.8565	1.0999	0.6910	1.6468	0..9059	1.0448	0.3818
GCV (%)	16	3	12	12	7	12	14	19
h^2_A	0.186	0.081	0.292	0.354	0.191	0.293	0.267	0.477

Table 8. Adjusted general combining ability effects

Parents	Traits							
	100.SW	**CN**	**H.6**	**h.6**	**DRC**	**NBAL**	**TNB**	**LS**
2	-0.217	-0.154	-0.236	0.004	-0.122	0.210	0.147	-0.047
3	-0.673*	-0.026	-1.869***	-0.697	-0.076	-0.106	-0.355	-0.205
45	0.127	0.107	-2.627***	-1.358***	-0.330	-0.215	-0.006	-0.041
50	1.449***	-0.185	-0.027	0.240	-0.161	-0.498**	-0.131	0.409*
205	0.552*	0.253	0.317	-0.260	0.503	0.209	0.108	0.398
206	-0.559*	0.045	0.985	0.760	-0.127	0.050	-0.292	-0.194
209	-2.266***	-0.020	-0.496	-0.393	-0.260	0.091	0.574*	0.311
x	2.002***	0.101	2.328***	1.192**	0.894***	-0.077	0.490*	-0.280
y	0.853**	-0.079	-0.143	-0.017	-0.220	-0.005	-0.342	-0.590**
z	-1.308***	-0.042	1.774**	0.535	-0.106	0.344*	-0.188	0.242
SE(g_i - g_j)	0.068	0.055	0.146	0.106	0.069	0.040	0.063	0.053

Table 9. Genetic correlations at GCA level (upper line) and phenotypic correlations (lower line with 43 degree of freedom)

	CN	H.6	h.6	DRC	NBAL	TNB	LS
100.SW	0.02	0.14	0.26	0.53	-0.72	-0.13	-0.24
	0.20	0.39*	0.41*	0.60***	-0.13	0.15	-0.33*
CN	-	0.03	-0.18	0.71	0.31	0.34	0.09
		0.13	-0.01	0.35*	0.16	0.13	0.04
H.6		-	0.95	0.56	0.32	0.13	0.10
			0.94***	0.67***	0.49***	0.42**	-0.13
h.6			-	0.50	0.11	0.03	-0.09
				0.58***	0.34*	0.32*	-0.20
DRC				-	0.03	0.38	0.04
					0.30*	0.56***	-0.19
NBAL					-	0.05	0.08
						0.31*	-0.07
TNB						-	0.40
							0.10

Table 10. Expected genetic gain (ΔG) for the main traits

Traits	ΔG (%) if the best 10, 20, 30, 40 of the 90 families were selected			
	10	20	30	40
H.6	6.0	4.7	3.9	3.1
h.6	8.9	7.1	5.8	4.7
DRC	2.7	2.1	1.7	1.4
LS	17.4	13.7	11.2	9.1

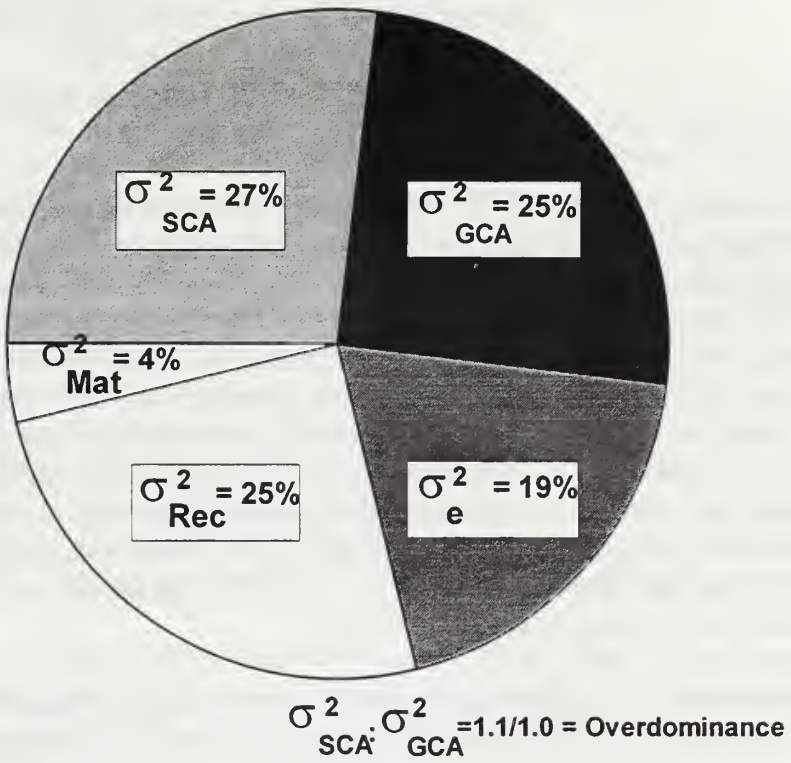

Fig. 2 - The structure of the phenotypic variance for total height

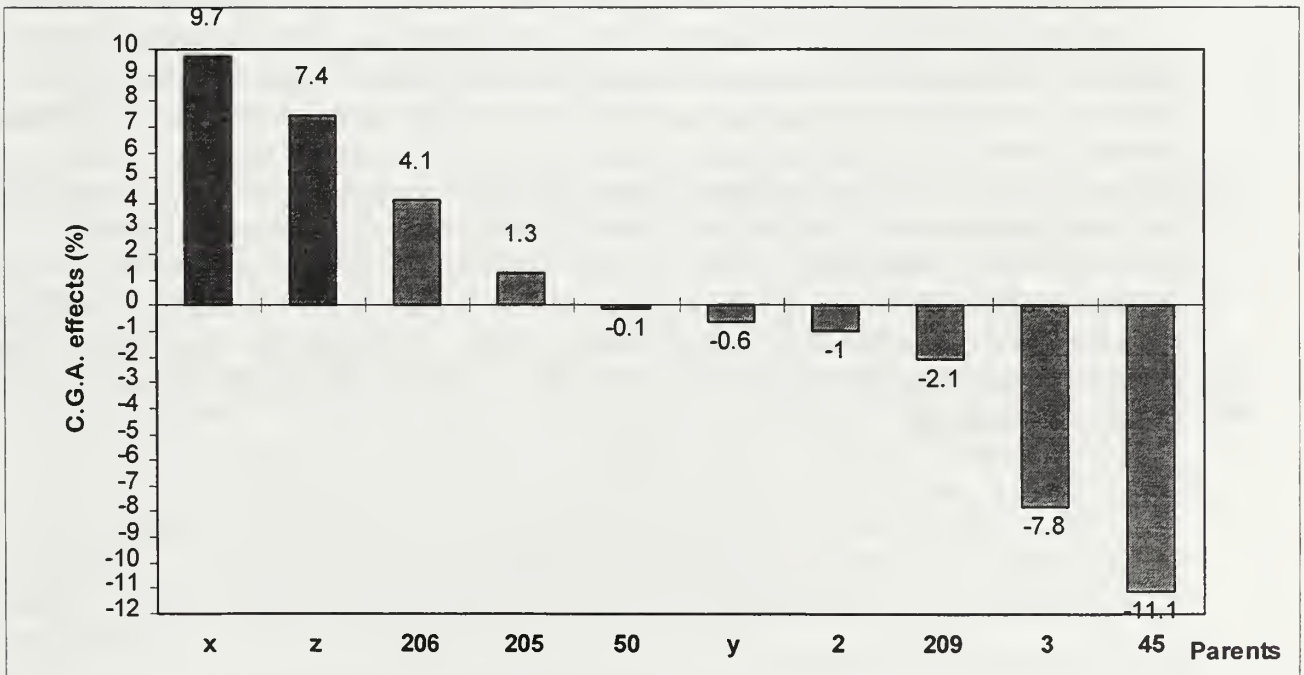

Fig. 3 - Adjusted general combining ability effects (%) for total height growth (H.6) at age 6

PROTECTION OF INDIVIDUAL TREES IN PINE SEED ORCHARDS FROM ATTACKS BY CONE AND SEED INSECTS

G. L. DeBarr and L. R. Barber[1]

Abstract: Two approaches for protecting individual trees are presented. A Single Tree Spray System consisting of irrigation spray nozzles permanently mounted on PVC pipes was used to apply insecticides in two seed orchards. White pine cone beetles, and the leaffooted and shieldbacked pine seedbugs were controlled by Asana XL®, esfenvalerate, in an eastern white pine seed orchard in western North Carolina. A second installation of the Single Tree Spray System was used to apply Guthion®, azinphosmethyl, which also reduced cone attacks by webbing coneworms on loblolly pines in a seed orchard in eastern North Carolina. Trunk implants of Orthene®, acephate, a systemic insecticide, protected individual loblolly pines from attacks by coneworms and seedbugs in a loblolly pine seed orchard in central Georgia. Criteria such as controlled breeding operations, genetic value, cone crop size, and inherent susceptibility to attacks can affect the need for protection and the allocation of control efforts for cone and seed insect pests on individual orchard trees.

Keywords: *Conophthorus coniperda, Dioryctria* spp., *Leptoglossus corculus, Pinus strobus* L., *Pinus taeda* L., *Tetyra bipunctata*, acephate, Asana XL®, esfenvalerate, Guthion®, azinphosmethyl.

INTRODUCTION

As advanced generation seed orchards become productive, older orchards are less intensively managed. However, orchard managers often continue to harvest seed or make expensive controlled pollination's on ramets of the best clones in older orchards. Such situations create a dilemma for orchard managers trying to protect a few selected trees from attacks by cone and seed insects. Aerial applications of registered insecticides will protect these scattered trees, but this approach is costly and inefficient. Individual trees can be protected by applying insecticides with conventional ground equipment (Nord et al. 1984), but good spray coverage on tall trees is often difficult or impossible to achieve. Our objective was to evaluate the efficacy of two alternative approaches to protecting individual trees: 1) a Single Tree Spray System using permanently mounted spray nozzles on PVC pipes; 2) a single annual implant of the systemic insecticide, Orthene®.

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MATERIALS AND METHODS

Single Tree Spray System The Single Tree Spray System was installed at two seed orchards. Installation and operation of the system is described in detail by Kilroy et al. (1996). The first installation was on eastern white pines, *Pinus strobus* L., at the USFS Beech Creek seed orchard near Murphy, NC. Thirty trees 45-50 ft. in height were selected for the study. Two treatments (sprayed or unsprayed) were randomly assigned to pairs of ramets from 15 clones. Ten sample branches with a total of 100 or more cones were tagged on each tree in late March, 1995. Each tree was sprayed with five gallons of 0.025% AI Asana XL® on March 16, April 6, and June 6. The number of healthy cones, cones killed by the white pine cone beetle, *Conophthorus coniperda* (Schwarz), and cones infested by the white pine coneborer, *Eucosma tocullionana* Heinrich, were counted on the sample branches in June. Ten apparently healthy cones were collected from each study tree in August. The seed were extracted, x-rayed, and the numbers of seed bug-aborted seed and seed bug-damaged caused by the southern pine seed bug, *Leptoglossus corculus* L., and the shieldbacked pine seed bug, *Tetyra bipunctata* (H.-S), were counted on the radiographs.

The second Single Tree Spray System installation was on loblolly pines, *Pinus taeda* L., at the Weyerhaeuser seed orchard near Washington, NC. Forty-five trees 50-60 ft. in height were used for the study. Three treatments (sprayed 1995, sprayed 1995 & 1996, and unsprayed) were randomly assigned to ramets from 15 clones. Each tree was sprayed with 5 gal. of 0.2 % Guthion® on April 21 and June 1, 1995 and 10 gal. on April 19 and June 5, 1996. Peak pollen shed occurred on April 10, 1995 and April 12 and 13, 1996. The number of cones killed by the webbing coneworm, *Dioryctria disclusa* Heinrich, or other *Dioryctria* spp., and number of healthy cones on the south 1/2 of each tree crown were counted during June of each year. Spraying was discontinued in 1996 because of severe damage to the trees and the Single Tree Spray System caused by hurricane Fran.

Orthene® Implants Implants of Orthene® systemic insecticide were made at the Weyerhaeuser seed orchard near Lyons, GA. Thirty loblolly pines 40-45 ft. in height were selected for the study. Three treatments (Orthene® - Feb., Orthene® - April, or unsprayed) were randomly assigned to ramets from 10 clones. Holes ca. 4 inches deep and 1/2 inch in dia. were drilled at a spacing of 5 inches around the circumference of the bole of each tree at a height of about 4 ft. above ground line. Each hole was filled with 1/3 fl. oz. (10 ml) of a saturated water solution containing 604 g of dissolved Orthene® 75S per liter. Implants were made on February 22 and April 22, 1996. Holes were drilled in untreated trees and filled with distilled water. Numbers of cones killed by *Dioryctria* spp. in the spring (small dead cones) and the summer (large dead or infested cones), and the number of healthy cones were counted on the south 1/2 of each tree crown during September. Seed extracted from ten apparently healthy cones per tree were x-rayed, and the number of filled, empty, seed bug-aborted, and seed bug-damaged seed were counted on the radiographs.

All data were analyzed by the GLM procedure and Dunnett's or Duncan's tests were used to detect significant differences among treatments at the $\alpha = 0.10$ probability level (SAS 1988). Percentage data were transformed using the arcsine $\sqrt{\%}$ or the log (x+1) transformations.

RESULTS AND DISCUSSION

Single Tree Spray System Eastern white pines protected by Asana® applied with the Single Tree Spray System had significantly fewer cones killed by the white pine cone beetle, cones infested by coneborers, and more healthy cones (Table 1). Previous attempts to control the white pine cone beetle by spraying insecticides have failed (DeBarr et al. 1982). This the first demonstration of successful control of the white pine cone beetle by spraying insecticides.

Table 1. Mean percentage of cone beetle-killed, coneborer-infested, and healthy cones on eastern white pines protected with ASANA XL® applied with the Single Tree Spray system, Beech Creek Seed Orchard, Murphy, NC, June 1995.

	Treatment	
Cone condition	Unsprayed	Sprayed
Killed by cone beetles	36.5a	19.7b
Infested by coneborers	1.5a	0.6b
Healthy	62.0a	79.7b

Means followed by the same letter are not significantly different (α=0.1) Dunnett's one-tailed t-test (SAS 1988).

Seed samples from sprayed eastern white pines had lower percentages empty seed, seed bug-damaged seed, and aborted seed caused by seed bugs than unsprayed trees (Table 2). Cones from sprayed trees also had higher percentages of filled seed and yielded significantly more filled seed per cone.

Loblolly pines protected by Guthion® applied with the Single Tree Spray System had significantly lower percentages of cones killed by the webbing coneworm than unsprayed trees in 1996 (Table 3). Peak pollen shed occurred on April 12 and 13 during 1996 and the trees were sprayed on April 19. This application date is within the 7 day "window of opportunity" for killing webbing coneworm larvae as they exit the pollen catkins and attack cones (G. L. DeBarr and L. R. Barber -- unpublished data). The April 21, 1995 application in date was 11 days after peak pollen shed and cone attacks by the webbing coneworm larvae had already occurred. Trees sprayed both years had significantly higher percentages of healthy cones than unsprayed trees.

Table 2. Average seed quality and yield per cone on eastern white pines protected with ASANA XL® applied with the Single Tree Spray System, Beech Creek Seed Orchard, June 1995.

Treatment	Seed quality				Seed yields
	% Filled	%Empty	% Seed bug-aborted	% Seed bug-damaged	No. filled seed/cone
Unsprayed	73.0a	18.8a	1.6a	5.0a	37.6a
Sprayed	88.5b	8.7b	0.5b	1.4b	53.5b

Means followed by the same letter are not significantly different (α=0.1) Dunnett's one-tailed t-test (SAS 1988).

Table 3. Mean percentages of webbing coneworm-killed and healthy cones on loblolly pines protected by ASANA XL® applied with the Single Tree Spray System, Weyerhaeuser Seed Orchard, Washington, NC, June 1995.

Cone condition	Treatment		
	Unsprayed	Sprayed 1996	Sprayed 1995 & 1996
Killed by webbing coneworm	6.9a	2.7b	2.3b
Healthy	88.9a	92.0ab	95.6b

Means followed by the same letter are not significantly different (α=0.1) Duncan's Multiple Range Test (SAS 1988).

Orthene® Implants Loblolly pines protected with implants of Orthene® made in February or April had significantly lower percentages of spring and early summer attacks by *Dioryctria* spp. than unsprayed trees (Table 4). However, only the April implants of Orthene® had significantly lower percentages of late summer attacks than unsprayed trees. This suggests that the February implants were too early to provide protection from coneworms for the entire summer.

The numbers of seed bug-aborted seed in cones from trees implanted with Orthene® in February or April were significantly lower than those in cones from unsprayed trees (Table 5). However, the implants did not significantly reduce the numbers of seed bug-damaged seed. Seed bug aborted seed are caused by the leaffooted pine seed bug feeding on developing seed in cones during late May through June. In contrast, seed bug-damaged seed detectable on the radiographs

are caused by leaffooted and shieldbacked pine seed bugs feeding on maturing seed in August and early September. These results suggest that implants of Orthene® provided control of seed bugs during the spring and early summer, but were ineffective in preventing late summer damage.

Table 4. Mean percentages of cones killed by coneworms on loblolly pines protected with trunk implants of Orthene® systemic insecticide, Weyerhaeuser Seed Orchard, Lyons, GA, 1996.

	Treatment		
Attack period	Unsprayed	Orthene® (Feb.)	Orthene® (Apr.)
Spring & early summer	2.9a	0.8b	0.7b
Late summer	7.3a	6.5ab	3.6b
Totals	10.2a	7.3ab	4.3b

Means followed by the same letter are not significantly different (α=0.1) Duncan's Multiple Range Test (SAS 1988).

Table 5. Mean numbers of seed bug-aborted seed and seed bug-damaged per cone on loblolly pines protected with trunk implants of Orthene® systemic insecticide, Weyerhaeuser Seed Orchard, Lyons, GA, 1996.

	Treatment		
Seed condition	Unsprayed	Orthene® (Feb.)	Orthene® (Apr.)
Seed bug-aborted	3.4a	0.9b	0.5b
Seed bug-damaged	6.0ab	4.1a	8.1b

Means followed by the same letter are not significantly different (α=0.1) Duncan's Multiple Range Test (SAS 1988).

The idea of using fixed-pipe sprayers to control cone and seed insects in tall trees is not new. Early workers (Grigsby 1964, Ciesla and McConnell 1965, Ciesla et al. 1967), tried to control cone and seed insects on southern pines using fixed-pipe systems. More recently this

technique was tried on conifers in the western United States (Personal Communication with Dr. Nancy Rappaport, USFS, Pacific S.W. Res. Sta., Davis, CA). However, our tests are the first to demonstrate efficacy of a Single Tree Spray System. This approached can be used by orchard managers to apply any of the insecticides currently registered for use in seed orchards. The feasibility of using systemic implants for cone and seed insect control was also demonstrated many years ago (Merkel and DeBarr 1974). Our results suggest that Orthene® implants have potential for cone and seed insect control on loblolly pines. However, this insecticide use pattern must be registered by EPA before it can be used operationally.

DeBarr (1971) suggested that managers consider protecting individual trees in seed orchards. However, in the past such an approach was unacceptable to mangers because all the seed from operational orchards had value and were harvested. Today there are situations where the protection of individual trees is desirable. Criteria such as controlled breeding operations, genetic value, cone crop size, and inherent susceptibility to attacks can affect the need for protection and the allocation of control efforts for cone and seed insect pests on individual orchard trees.

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LODGEPOLE PINE FROM FAR NORTHERN CANADA PERFORMS WELL IN A MILDER SWEDISH ENVIRONMENT

Tore Ericsson[1]

Abstract. Results of a height assessment made in a 12-year-old experimental plantation at latitude 62° N, altitude 550 m in Sweden indicate that the productivity of lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) from the very far north of its distribution range in western Canada was generally good. Only small differences in average performance could be ascribed to variation in the geographical origin of 801 open-pollinated, single-tree progenies collected at latitudes ranging from 51 to 64° N and altitudes from 400 to 1700 m in British Columbia and the Yukon Territory. Performance, predicted based on parent breeding values for tree height, decreased by an average of 8 % when moving from the lowest to the highest altitudes of origin, whereas when moving from the southernmost to the northernmost site of origin it increased slightly. An analysis in which latitude and altitude were simultaneously taken into account confirmed the greater importance of altitudinal origin, although this conclusion should be interpreted with care since the latitudes and altitudes were strongly correlated ($r = -0.73$). The experimental site was situated in the southern part of the north-Swedish lodgepole pine utilization area. Thus the findings suggest that the use of lodgepole pine from the northernmost seed importation locations may be profitable even in comparatively southern Swedish reforestation zones. Furthermore, such provenances should have the added advantage of being less susceptible to environmental stress. Where there is concern about the ability of lodgepole pine to tolerate harsh conditions in southern zones, these results suggest that it would be better to use more northern provenances rather than provenances from higher elevations.

Keywords: Altitude, breeding value, half-sib, hardiness, height, latitude, open pollination, *Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm., provenance, survival, Sweden.

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INTRODUCTION

During the early introduction of lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) to Sweden, more northern provenances were sometimes suspected to have equally high production potential on sites where more southerly provenances had so far been assumed to be 'optimal' as discussed by Lindgren et al. (1988). Since then, a shift towards more northern provenances has often been suggested (e.g. Lindgren et al. 1993) though without much further experimental basis. In order to bring to light details which might support or refute such hypotheses, some test sites within the southern part of the geographical utilization range in Sweden were planted with both very northern and more southern provenances in the spring of 1984.

MATERIAL AND METHODS

This report examines one of the 1984 test sites[2], where roughly 850 open-pollinated single-tree progenies (assumed half-sibs), representing around 70 stands, were tested. Their origin was the interior distribution region of lodgepole pine in Canada between 50°51' and 63°52' N. As shown in Fig. 1 (further described by Ericsson 1993), they represented most families collected in the 1970s

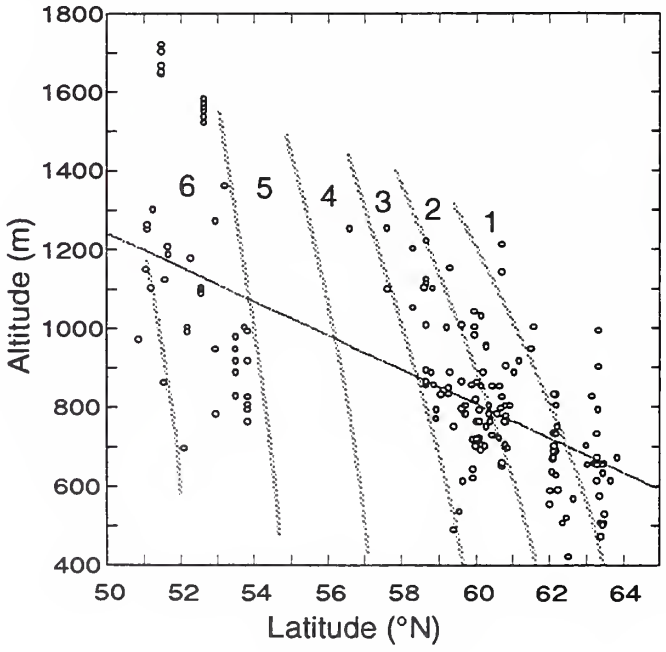

Fig. 1. The distribution of origins of 801 examined open-pollinated families. Seed collection zones 1–6 and the regression line of altitude on latitude ($r = -0.73$) are indicated. Points are frequently overlaid.

[2]Identification at SkogForsk: S23F846383 Dalsvallen

from the lodgepole pine selection zones 1, 2, 3, and 6. Zone 1 to 6 correspond to the span from the northern Yukon south to central British Columbia. The test site, situated on 62°11' N, 13°35' E at 550 m elevation in Sweden, is actually located around where the zone 4 or 5 material was intended to be used, but those zones were not represented. (Among provenances, interpolation was to be preferred before extrapolation. Other test site results presented later will complement the information on material from all zones.) Despite the exclusion of zones 4 and 5, the huge number of progenies was considered technically impossible to handle within one single experimental area. Therefore, the material was subdivided into six sets, each of which was designed to hold, as far as possible, equal representation of mother trees selected within a stand or neighbouring stands.

Thus six independent experiments were installed close to each other, each containing equivalent provenance samples. An individual family was usually represented by ten seedlings distributed randomly within a 'single-tree plot' experiment, arranged with 1×2.2 m spacing. Survival data and heights from an average of about nine living trees per family were collected in September 1995 (Table 1). With respect to the provenance investigation, the equivalent composition of the six experiments did legitimate that data and results were merged.

Table 1. Mean tree survival and height in six equivalent 12-year-old provenance experiments on one test site.

Experiment no.	Number of		Survival (%)	Mean height (cm)
	half-sib families	living trees		
1	133	1238	91.8	260.7
2	134	1301	92.8	238.9
3	133	1348	94.2	238.1
4	137	1338	93.7	227.7
5	133	1230	92.1	215.0
6	131	1269	93.8	226.4
Sum / mean	801 [a]	7724	93.1	234.5

[a] Totally 53 families with less than five trees were discarded

Breeding values for tree height were separately computed within each experiment, where the fixed effects of the mixed-model equations corresponded to a ten-block area subdivision, designed to eliminate disturbing environmental influence (cf. Ericsson 1997). The mother tree breeding values from all six sets were subsequently merged and chosen to represent the general production potential to be compared by provenance. The mean of heritability estimates for tree height was $h^2 = 0.209$.

Survivals were compared using actual occurrences.

RESULTS AND DISCUSSION

The association between productivity and origin was examined by linear regressions of tree height, (h cm) on latitude and altitude of origin (l ° and a m) respectively (Fig. 2). The average influence of the latitudinal origin was quite small and in favour of northern seed source origins ($h = 198.5 + 0.6171l$; Fig. 2a). However, the influence of seed source altitude was stronger ($h = 247.8 - 1.511a/100$; Fig 2b), favouring milder climates. The height increased by an average of 8 % when moving from the highest (1700 m) to the lowest altitude of origin (400 m), while the corresponding height difference between extreme latitudes was 3 %.

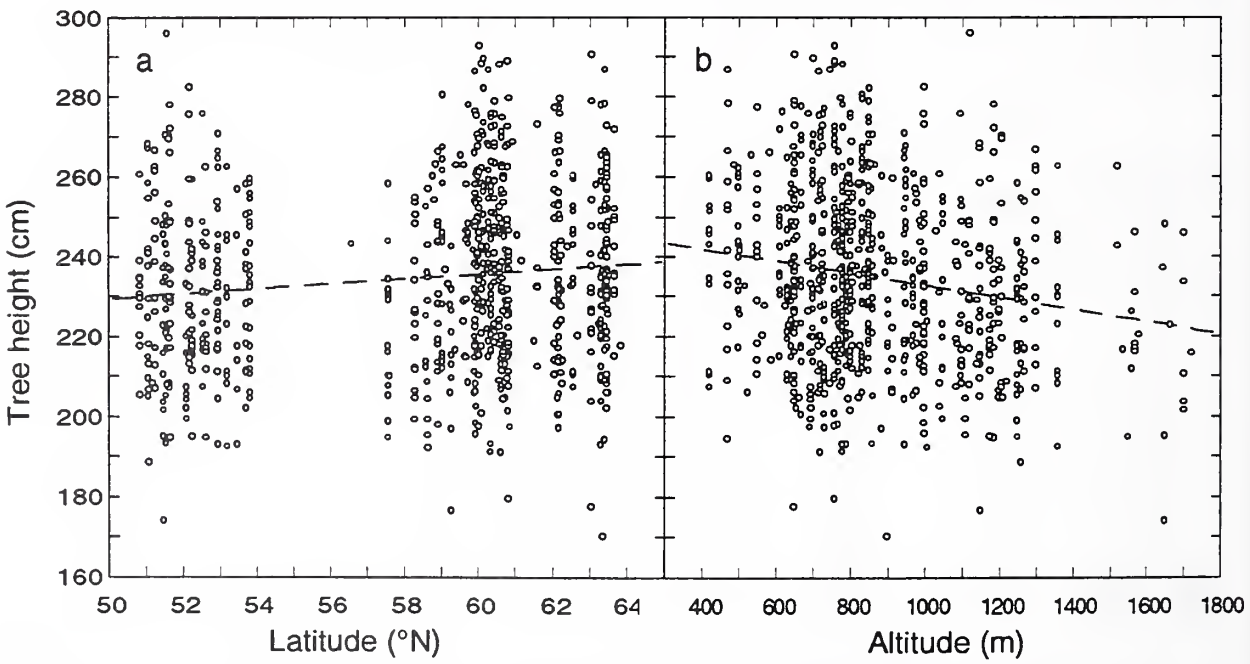

Fig. 2a–b. Mother tree breeding values for height plotted against mother tree latitude and altitude, respectively. The broken lines indicate regression association.

A multiple regression on both latitude and altitude removed most influence from latitude and added a little more influence to altitude ($h = 253.8 - 0.08575l - 1.617a/100$; not shown). Thus, the regression analysis appears to indicate that the latitude of origin is relatively unimportant compared to altitude. One must however recognize the strong correlation between them (Fig. 1), which makes such a conclusion somewhat uncertain.

The tree mortality rate had been very low up to the time of assessment, and in many families all trees had survived. Therefore, survival plotted in the same way as is height in Fig. 2, will be difficult to interpret because of the aggregation at 100 %. Instead survival class means are displayed jointly with mean heights in Fig. 3, using classes corresponding to seed collection zone, latitudinal, and altitudinal subdivisions respectively (cf. Fig. 1). Although the differences were small, the northern and/or low-elevation progenies showed less mortality. The height bars are class means from the data of Figs. 1 and 2. The visible positive correlation between survival and height in Fig. 3 is a consequence of the class subdivision. It turns out quite small when computed from family data (0.02).

Lindgren & Nilsson (1992) found a clear population-mean differentiation by latitude in the development of frost tolerance of needles from five-year-old trees originating from roughly the same provenance range considered for Sweden, from 55° N in central British Columbia to 63° N in the Yukon Territory. The results of this investigation support the use of furthest northern origins to obtain increased hardiness on north Swedish sites without loss of productivity. The lower productivity of high-altitude provenances, mentioned by Rehfeldt (1980, 1983) and coinciding with Fig. 3c, is thus avoided. The results likewise support the suggestion by Fries & Lindgren (1986), among others, that higher latitude origins rather than higher elevations will increase hardiness with a minimum loss of productivity.

These findings indicate that a few northern, outstanding provenances may be sufficient for use all over northern Sweden, in accordance with the conclusion by Lindgren (1993) that single provenances have been noted to perform surprisingly well over a wide geographical range. A natural question is whether this would also be a possible outcome in Canada. Southward transfer there is normally associated with notable slower growth, naturally accompanied by better survival. Ying et al. (1985) show repeatedly negative correlations between height and latitudinal as well as elevational origin. On the other hand, they note that suitable provenances from interior sources may grow quickly and appear to be broadly adapted to diverse site conditions. The elevational influence on population differentiation seems to decrease with increase in latitude (Ying 1991). Nothing indicates, however, that the latitudinal differentiation would be relatively lower on higher latitudes in Canada.

It is unclear whether or not such flexibility of northern lodgepole pine would occur in Canada if, for example, Yukon provenances were utilized in central interior British Columbia. If there are

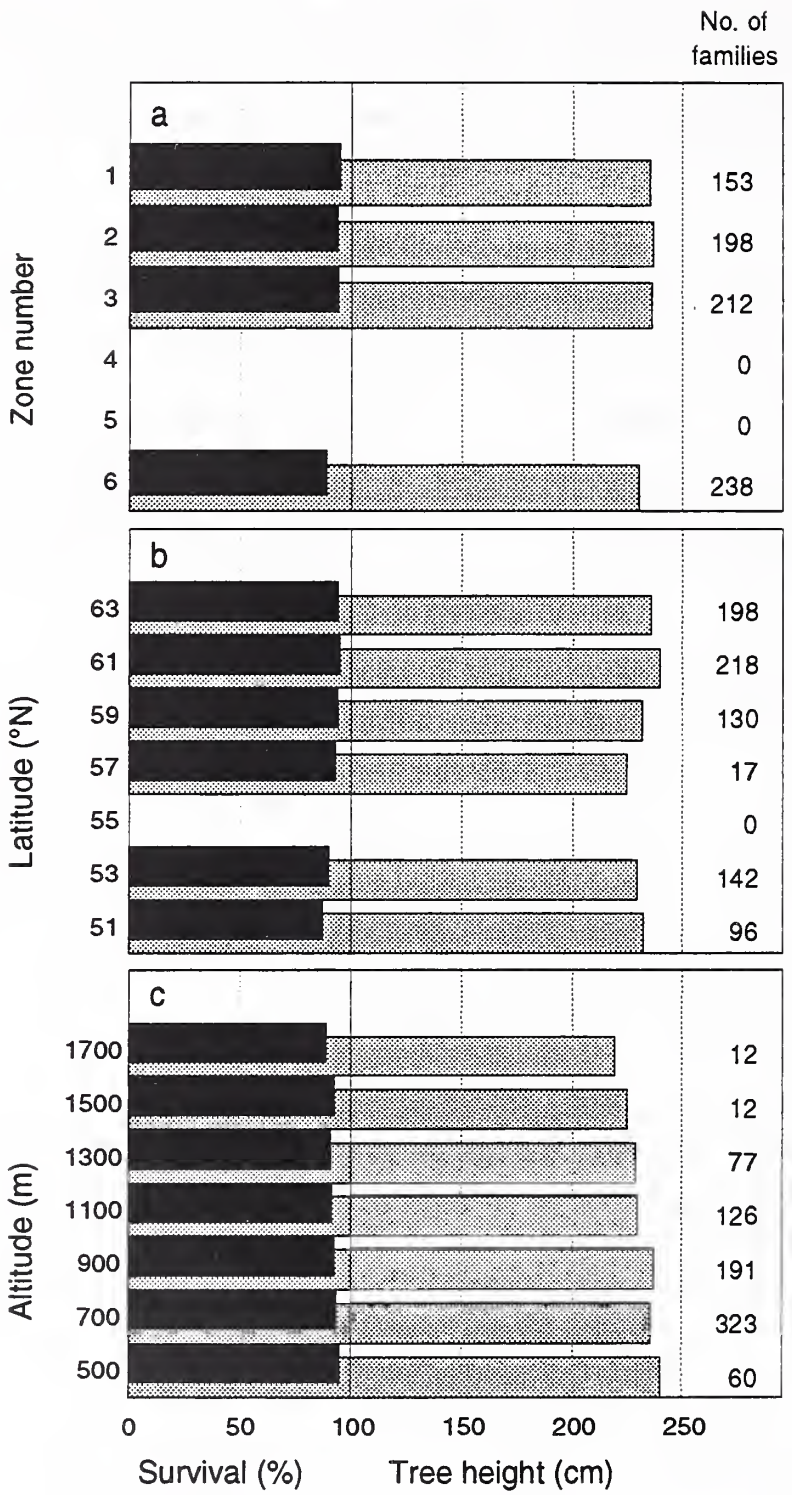

Fig. 3a–c. Mean survival and mean tree height from mother tree breeding values classified by seed collection zone and by latitude and altitude class of the mother trees (middle class values indicated).

differences compared to Sweden they may be due to differences in light, which is latitude-dependent and little modified by weather fluctuations. The typical transfer of lodgepole pine from Canada to Sweden implies a five-degree-move northward in order to obtain a roughly equivalent temperature climate. Thus the light environment is different between Canada and Sweden on otherwise similar sites.

CONCLUSIONS

The results indicate that the recommendations regarding lodgepole pine provenance choice for north Swedish reforestation sites may be revised. More northern provenances than previously suggested (Lindgren et al. 1988) should be considered, if available. Such a choice seems to combine equal production potential with better robustness with respect to climatic stress, leading to lower mortality rates.

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THE LONG-INTERNODE BREED OF RADIATA PINE - A CASE STUDY OF BREED DIFFERENTIATION

K.J.S. Jayawickrama[1], C.J.A. Shelbourne[1], M.J. Carson[1] and P.A. Jefferson[1]

Abstract: Development of the long-internode breed of radiata pine in New Zealand is an example of genetic differentiation within a forest tree species to obtain a specific end-product. Radiata pine shows marked variation in the number of branch clusters formed annually, with a corresponding variation in internode length. Long internodes are commercially valuable due to greater yield of knot-free lumber in the unpruned part of the stem for a variety of products. Internode length is under strong genetic control and is highly amenable to selection. Recent sawing studies of a 28 year-old clonal test have shown that clones selected for long internodes gave much higher lumber value as US shop grades.

Breeding for internode length began in 1970 with the selection of 104 long-internode plus trees in plantations. Subsequent work includes open-pollinated progeny testing of both the first-generation selections and 74 second-generation selections, 153 crosses involving the first- and second-generation selections, and further first-generation selections in 1985. Advanced-generation selections will be made in the control-pollinated trials in 1998.

Gains in internode length entail somewhat reduced gain in the following: volume growth, branch size and stem quality. Problems in stem form are greater on highly fertile or very exposed sites. The adverse genetic correlations have led to separation of the long-internode population from the main breeding population. The goal is a tree with one or two branch clusters per year, to be grown on specific sites, with suitable silviculture. Deployment of improved stock is through control-pollinated seed of full-sib families (with the option of vegetative amplification). A clonal forestry option also exists, and is becoming more attractive as vegetative multiplication becomes less expensive.

The next major advance for the long-internode breed will be forming 2 small elite sublines of about 12 individuals each. These will have the best of the 3 distinct genetic resources available. Internode length will remain a major emphasis, with threshold values imposed for growth rate, stem form, resistance to Cyclaneusma needlecast, wood stiffness and spiral-grain angle. Future breeding will largely be within the sublines, allowing for unrelated crosses between sublines for production purposes.

Keywords: clear lumber, branch cluster frequency, Pinus radiata D. Don

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INTRODUCTION

Radiata pine (Pinus radiata D. Don) is the main commercial forestry species in New Zealand. Differentiation of tree types in terms of internode lengths has long been seen as desirable to accommodate different products (Shelbourne 1970; Carson and Inglis 1988) These include boards, structural timber, veneers, posts and poles, panel products, reconstituted wood products, kraft and mechanical pulp and paper (Carson and Inglis 1988; Kininmonth and Whiteside 1990).

Genetic improvement of radiata pine in New Zealand began in 1953 with selection of first-generation plus trees in plantations (Shelbourne et al. 1986). Early emphasis was on height, diameter, straightness, lack of malformation, small branch size and freedom from stem cones. It was seen that radiata pine varies markedly in branch cluster frequency (the number of branch clusters, from 1 to 6, formed annually), with a corresponding variation in internode length. This trait proved to be highly heritable (Bannister 1962). The major advantage of long-internode trees is in the production of usable lengths of clearwood in unpruned logs (Carson and Inglis 1988).

The main breeding programme for radiata pine, with selection for fast-growing, well-formed trees with light, wide-angled branches, provides trees suitable for structural timber (framing, etc) and, when pruned, long clear timber. This population is the main population or the 'Growth and Form' breed in Carson and Inglis (1988) and Carson et al. (1990). However the selection has also driven internode length below that of unimproved plantations (Carson and Inglis 1988), which will have a marked, deleterious effect on the production of internodal clearwood. By 1970, there seemed to be justification for development of a 'long-internode' breed of radiata pine. This tree type was to meet the need for boards, short clears for remanufacture and clear veneers from short peeler bolts (Shelbourne 1970). Differentiated breeds (populations) were later formed for Dothistroma resistance and higher wood density (Carson et al., 1990).

Influential work by Fenton and Sutton (1968) helped promote the 'direct sawlog regime'. This used wide spacing, heavy thinning and pruning to achieve maximum clearwood production over a rotation of 25-35 years. This intensive silviculture, combined with good growing conditions and genetic improvement have contributed to rapid tree growth and greatly improved stem form in the radiata pine plantations in New Zealand. Plantation areas have increased dramatically (now 1.4 million hectares for P. radiata) but rigorous investment criteria have encouraged the harvest of ever-younger trees, as early as 20 years in one account (Macalister 1997). However very young trees have a high proportion of juvenile wood with its attendant drawbacks, such as greater twist, lower density, lower strength and poorer lumber recovery (Cown 1992). Wood quality problems are aggravated on highly fertile ex-pasture sites (Macalister 1997). There is serious concern as how an increased proportion of juvenile wood will affect New Zealand's forest products industry (Cown 1992, Macalister 1997).

During the 1990s the NZ tree improvement focus has changed from improving growth and log quality to improving specific end-product values. The tree breeder's task now adds improving juvenile wood quality (particularly stiffness and stability), to further improving a breed for increased clearcuttings. More and more plantations are being established as full-sib family blocks, and clonal forestry is being developed. It is likely that forest trees will be increasingly differentiated into 'breeds' just as agricultural and fruit crops have been differentiated into cultivars. The long-internode breed of radiata pine provides a useful case study of breed differentiation for a forest tree species.

FORMING THE LONG-INTERNODE BREED

Over 150 plus-trees with the long internode habit were selected in plantations between 1970 and 1973 (Shelbourne et al. 1986). Open-pollinated families of these selections were established as an initial breeding population. These progenies have yielded important information on the genetic control of this trait. Seventy-four second-generation selections were made in 1982 within the open-pollinated progeny tests of these first-generation selections. A further 52 first-generation long-internode selections were made in 1985. Open-pollinated seed were collected from these 52 clones to establish progeny tests (jointly with progeny of the second-generation selections) on two sites in 1987. Control-pollinated trials with 153 crosses (involving first- and second- generation clones) were planted on two sites in 1990 and will be measured shortly.

The best first- and second-generation selections remain in clonal archives. Thus the genetic resource of the long-internode breed now includes over 50 first-generation and 37 second generation selections and potential third-generation selections. For reasons described later, this population has been kept separate from the mainline (largely multinodal) breeding population.

THE MEASUREMENT AND GENETICS OF INTERNODE LENGTH

Several methods have been used to assess the long-internode trait. The most direct (but also the most time-consuming) is to measure the distances between the zones of knots associated with successive branch clusters up the stem. From these data can be derived *mean internode length* and measures of frequency of long internodes such as *internode index* (the proportion of the log in internodes of 60cm or greater). This has usually been measured in the first and second logs, to 5.7 and 11.2 meters (18.8 and 37 feet) respectively. A direct count of the *number of branch clusters* is another useful quantitative measure. *Branch cluster frequency* or *branch habit* can be scored subjectively on a 1 to 9 scale; this is easy to estimate and can prove a reliable indication of family rankings for the long internode habit and of internode index. Branch cluster frequency is routinely assessed in genetic trials of radiata pine in New Zealand. Heritability estimates for branch cluster frequency are given in Table 1; it is often the most heritable of the growth and form traits. High heritabilities and coeffiecients of variation were also observed for branch cluster frequency in studies of the native populations (from USA and Mexico), grown in New Zealand (Burdon 1992).

Table 1. Heritability estimates from three open-pollinated radiata pine progeny tests established in the central North Island of New Zealand.

Genetic material	Age in years	Number of families	Branch cluster frequency score		DBH		Straightness score	
			h^2_i	h^2_{hs}	h^2_i	h^2_{hs}	h^2_i	h^2_{hs}
1st generation selections[1] ("885 series")	8	467	0.33	0.67	0.21	0.56	0.21	0.56
1st gen. long-internode[2] selections ("870 series")	10	104	0.27	0.65	0.26	0.66	0.20	0.63
2nd gen. long-internode selections[1] ("883 series")	8	73	0.32	0.66	0.21	0.55	0.30	0.64

1 From unpublished data of K.J.S. Jayawickrama. Estimates based on one location.
2 From unpublished data of M.J. Carson, C.J.A. Shelbourne and C.B. Low. Estimates based on one location.
h^2_i = narrow-sense heritability on individual-tree basis, h^2_{hs} = heritability of half-sib family means

Differences in internode length between contrasting genotypes can be dramatic. The data in Table 2 are for progeny of open-pollinated highly-ranked first-generation long-internode selections, when compared with seedlings obtained from a first-generation 'Growth and Form' orchard. The largest difference was in the percentage of internodes above a given length. For example, while 45% of internodes exceeded 120 cm (48 inches) for the long-internode families, only 14% of internodes of the seed orchard progeny exceeded this length. Everything else being equal, these differences equate to large differences in the yield of long clear-cuttings.

Similar dramatic results were shown in a 12-year-old genetic gain trial with replicated block plots of 64 trees (Table 3). While controlled crosses among 'Growth and Form' trees had increased branch cluster frequency (by 1.9 points) compared with climbing select material, controlled crosses among long-internode trees had decreased branch cluster frequency by 3.0 points (a difference between the two breeds of 4.9 points). Considered in isolation, branch cluster frequency in radiata pine is a perfect trait for genetic manipulation - highly heritable, easy to assess and of major economic value.

Table 2. Comparison of the highest ranked 1st generation long-internode selections with 1st-generation 'Growth and Form' seed orchard seedlot[3].

	Dbh in cm (and inches)	Height in meters (and feet)	Straight-ness (units)	Mean internode length in cm (and inches)	% of internodes exceeding 60 cm (24")	% of internodes exceeding 120 cm (48")
Long internode progenies[1,2]	26.2 (10.5)	16.4 (54.1)	5.94	67 (26.8)	72	45
1st generation seed orchard mix[2]	25.9 (10.4)	16.1 (53.1)	6.50	47 (18.8)	44	14

1 8 of 100 open-pollinated families, selected for internode length
2 32 trees measured
3 from unpublished data of C.J.A. Shelbourne and D. Briscoe. Trees were measured at age 10 in an open-pollinated progeny test in the central North Island.

Table 3. Realized genetic gain for different seedlots, compared to climbing select (=seed tree) planting stock[1].

Genetic material	Branch cluster frequency[2]		DBH		Straightness		% Acceptable crop trees	
	score (1-9)	Change (units)	cm (inch)	Gain (%)	score	Change (units)	Mean	Change
C.P. crosses between 1st gen. ("870") long-internode selections	2.38	- 3.00	27.1 (10.8)	1.2	5.57	+ 0.99	52	+ 25
C.P. crosses between 1st gen. GF selections	7.28	+ 1.90	28.2 (11.3)	5.2	6.75	+ 2.17	75	+ 48
O.P. orchard mix of best 25 1st gen. GF selections	6.33	+ 0.95	28.0 (11.2)	4.6	5.53	- 0.95	53	+ 26
Climbing select (=seed tree) seedlot	5.38		26.8 (10.7)		4.58		27	

1 Unpublished data from C.B. Low, NZFRI. Age 12 results from a trial planted in the central North Island. C.P. = control pollinated, O.P. = open pollinated. GF = Growth and Form.
2 Measured on a subjective scale (1=1 cluster per year, 9=highly multinodal).
3 Measured on a subjective scale (1=1 very crooked, 9=perfectly straight).

Despite the ease of manipulation of internode length, there are certain disadvantages associated with the long-internode habit. Examples of genetic correlations between branch cluster frequency and two major traits (diameter and stem straightness) are given in Table 4. They show that families expressing the long internode habit tend to grow slower, be more crooked and have more malformation than the Growth and Form families. This is a common finding for genetic trials of radiata pine in New Zealand. For example, it can be seen from Table 3 that the long-internode control-pollinated families were less straight (1.2 points lower) and had a lower percentage of acceptable crop trees (52 vs 75) when compared to the GF control-pollinated families. Further, long-internode trees tend to have larger diameter branches and steeper branch angles than highly multinodal trees. While it is possible to select for well-formed trees with a long-internode habit, increasing the internode length does entail some reduction in gains for growth rate, stem form and malformation.

Two points should be noted when considering the growth and form of long-internode trees. First, the long-internode habit is in low frequency in the population and it is harder to find a long-internode tree with acceptable form and growth (Shelbourne 1970). Second, many more first-generation candidates showing average to high branch cluster frequency have been selected and tested in New Zealand (over 1500 compared to 200 long-internode selections) as they comprised the main breeding population. Thus the best parents in the main breeding population have come from a far larger pool of candidates compared to the best long-internode parents.

For this and other reasons the long-internode breed has not gained market acceptance in the past. Financial analysis using the growth and log-quality model STANDPAK showed that (given a range of price lists used and numerous, conservative assumptions) the Growth and Form breed usually gave the best return (Carson 1988). The abandonment of long-internode

seed orchards by the major seed company when under financial pressure meant that the supply of improved seed for the long-internode breed dwindled.

Recent realization by the forestry industry that the amount of clear cuttings from unpruned logs is much reduced in genetically improved stands has resulted in a renewal of interest in the long-internode breed, and in extending internode length in the main Growth and Form breed. Further, recent data show open-pollinated families of the best second generation long-internode clones to have comparable growth and form to families of good first-generation clones (grown on the same site), but a reduction of 1.5 units of branch cluster frequency score (Jayawickrama, unpublished data). In this case two cycles of selection within the long-internode population have achieved similar gains in growth and form to those from one cycle in the main population.

Table 4. Estimates of genetic correlation coefficients for branch cluster frequency, with three growth and form traits[1].

	Genetic correlation of branch cluster frequency[2] with		
Site	DBH	Straightness[3]	Malformation[4]
Woodhill	0.31	0.56	N.E.[5]
Maramarua	N.E[5]	0.46	0.31
Kaingaroa	0.57	0.51	0.38
Golden Downs	0.13	0.38	0.31
Eyrewell	0.33	0.34	N.E[5]
Berwick	0.22	0.17	N.E[5]

1 Measured in a *P. radiata* polycross progeny test on six New Zealand sites at age 9. From unpublished data by C.J.A. Shelbourne and C.B. Low. Reproduced with permission from Carson and Inglis (1988).
2 Scored on a subjective scale (1=one whorl per year, 9=highly multinodal).
3 Scored on a subjective scale (1= very crooked, 9=perfectly straight).
4 Scored on a subjective scale (1=highly malformed, 9=no malformation).
5 Not estimated due to imprecision of estimates of one or both variance components.

SITES, ESTABLISHMENT AND MANAGEMENT

Improved stock can be deployed through control-pollinated seed of full-sib families (with the option of vegetative amplification), and ultimately, through clonal forestry (Burdon 1991). If vegetative propagation is used, some physiological ageing (maturation) can be introduced. Such maturation can improve stem form in radiata pine (Menzies et al. 1991). Another approach being researched is to cross highly-ranked long-internode selections with Growth and Form selections showing outstanding form and fast growth.

There are strong site effects and management considerations in growing trees with long internodes. Trees grown on the central plateau of the North Island or at higher latitudes in the South Island tend to have longer internodes than on low-elevation sand-dune and clay soils in the northern North Island. Differences expressed among genotypes are greater on sites with good expression of the long-internode habit, and there appears to be little breed x site

interaction for internode length (Carson and Inglis 1988). Stand density appears to have minor effects on internode length (Grace and Carson 1993). However, long-internode genotypes grown on fertile sites at wide spacing tend to have poor form and to grow very large branches. Long-internode trees are also susceptible to top breakage in areas with frequent strong winds. Thus it is clear that the long-internode breed needs to be targeted to appropriate sites (i.e. certain sites in the South Island and less fertile sites in the central North Island) and given appropriate silviculture (Carson and Inglis 1988). A model has been developed to predict stand mean internode length for stands of both genetically improved and unimproved P. radiata (Grace and Carson 1993).

INTERNODE LENGTH, WOOD PROPERTIES AND UTILISATION

Trees from the Growth and Form breed tend to produce wood acceptable for lower-value framing timber or pulpwood from the unpruned part of the stem (second log and upwards), especially when grown at wide spacing. In contrast, long-internode trees are commercially valuable due to greater yield of knot-free lumber (short clears) in the unpruned part of the stem for a variety of industrial uses (e.g. random width boards for US millwork industry, veneer, appearance-grade clear cuttings, fingerjointings etc). This was shown, for example, in a recent sawing and wood-properties study involving ten 28-year-old clones with two trees per clone (Beaureguard, R., Gazo, R., Kimberley, M., Turner, J., Mitchell, S and Shelbourne, C.J.A., unpublished data). The clones were selected for a range of internode length, branch size, wood density, and diameter at breast height (dbh), and representative logs were sawn into random-width boards. All boards were graded by both Western (USA) Lumber Rules and New Zealand Appearance Grades, and (after resawing to framing), by NZ Visual Framing and Australian Machine Stress Grading Rules.

Broad-sense heritabilities for dbh, branch index (largest four branches per log) and internode index (percent internode lengths > 60 cm), spiral grain and density were over 0.9. The three long-internode clones showed values per m^3 that were 30-40% higher than the value of the other seven short-internode clones. These preliminary results, based on a very small number of clones, suggest that selection for high internode index can result in large gains in grade and value for US random-width boards and NZ Appearance Grade lumber.

By contrast, only minor differences were found in machine-stress framing-grade and value between multinodal and long-internode clones. At least for this material, it appeared that if branch size is controlled by relatively high stocking (350 stems/ha in this test), select clones of the long-internode breed could be used for structural timber, especially if also selected for clearwood stiffness.

FUTURE DEVELOPMENT OF THE LONG-INTERNODE BREED

The market demand for millwork lumber is predicted to remain, and recent studies have highlighted the product value associated with long-internode trees. The best second-generation selections appear to combine the long-internode habit with good growth and form. Pruning is costly in general and hard to effect on certain sites (e.g. steep areas), and major

forest owners are reassessing the economic rationale for pruning (Anonymous 1997). These factors can make growing of a long-internode tree cost-effective for some growers. The tree of choice will have good (but not extreme) expression of the long-internode habit, with acceptable growth, form and wood properties.

The next major advance of the long-internode breed will be the formation in 1998 of 2 small elite sublines of about 12 individuals each. These will contain the best of the 3 distinct genetic resources available (about 50 available first-generation clones, 37 second-generation clones, and the 153 full-sib families). Internode length will remain a major emphasis, with threshold values imposed for growth rate, stem form, resistance to Cyclaneusma needlecast, wood stiffness and spiral grain angle. Cyclaneusma needlecast can limit productivity on some sites, while radiata pine trees with high grain spirality produce unstable lumber. Clonal tests will be used in parallel with seedling progeny tests for estimation of breeding values. Crosses among the highly selected elite clones will be suitable for immediate use in the production population. Future breeding will largely be within the sublines, allowing for unrelated crosses between sublines for production purposes.

WHAT INSIGHTS CAN THE LONG INTERNODE BREED PROVIDE?

We feel the development of the long-internode breed of radiata pine can lead to several useful insights. First, the success of the programme is largely due to a long-term commitment (over 27 years) towards this goal, with only minor modifications along the way. Second, this goal of obtaining a specific end-product for clearly defined markets was slightly ahead of its time. In this case tree breeders anticipated, rather than catered to, industry requirements. Third, the differentiation of breeds is a logical progression in the intensity of management of forests. The progression is 1) natural stands 2) unimproved plantations 3) genetic improvement of growth rate, form and adaptability and 4) specific breeds for specific products.

Fourth, the differentiation of breeds is logical where adverse genetic correlations exist. In the case of radiata pine it is difficult to achieve rapid, simultaneous improvement of internode length, growth rate and stem form; some trade-offs are inevitable. For some end uses, the added value of knot-free lumber from the long-internode material can compensate for reduced gain in growth rate, branch size and straightness; for others it would not. Fifth, a strong knowledge base (genetic parameters, economic weights, etc) is needed to make the hard choices of what traits to optimize for a given product. Sixth, appropriate use of site and silviculture play an important role in optimizing the value this breed.

CONCLUSIONS

Differentiating radiata pine into breeds or clones selected for different end-uses is possible for a variety of end-products. The early development of a long-internode breed is one example. Markets and technologies can change enormously during the time it takes to grow a crop of this species and a "portfolio" of breeds and clones of known characteristics may

prove to be the most profitable in the future. However, improved juvenile wood is likely to be necessary for all purposes.

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TIMING OF NITROGEN APPLICATIONS IN A LOBLOLLY PINE SEED ORCHARD

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Abstract. To determine the optimal timing of ammonium nitrate fertilizer to stimulate female strobili production in loblolly pine (*Pinus taeda* L.) seed orchards, ammonium nitrate was applied on four different dates; April, June, July, and September in 1993, 1994, and 1995. The orchard was seven years old the first treatment year. Female strobili production increased throughout the study from an average of 40/tree in 1993 to 230/tree in 1995. Date of fertilizer application had very little affect on female strobili production in 1993 and 1994. Treatments made during the active growing season (April, June and July) resulted in equivalent number of female strobili. Following the 1995 treatment year, there were significantly fewer strobili only on trees fertilized late in the growing season (September). Differences among the 19 clones used in the study were huge and accounted for much of the variation in female strobili production. There was no clone by treatment interaction indicating that clones responded essentially the same to the different fertilizer treatments. The lack of fertilizer treatment differences suggests that applying ammonium nitrate virtually any time during the active growing season will stimulate flowering.

Keywords: *Pinus taeda* L., fertilization, female strobili.

Introduction

The application of fertilizers to enhance strobili production in southern pine seed orchards has become a route practice. It is used to enhance seed orchard development during the first four to five years following orchard establishment, to promote female strobili initiation during the "production" phase of the orchard, and to maintain health and vigor of the trees throughout the life of the orchard. Of the fertilizer elements routinely applied to seed orchards to stimulate strobili production, many published reports indicate that nitrogen is the most important or at least the most efficacious (Puritch 1972, Owens and Blake 1984, and Owens 1991). While nitrogen fertilization is widely used to stimulate strobili production, it is unknown whether nitrogen is important for induction, bud development, or the path leading to seed maturation (Bonnet-Masimbert and Webber 1995).

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Despite the lack of understanding, nitrogen fertilization remains a prevalent practice in operational seed orchards. However, only scant attention has been paid to the timing of nitrogen fertilization to maximize the female strobili response. Schmidtling (1975) reported that in a fertilization timing study in a fully mature loblolly pine seed orchard, fertilization with ammonium nitrate in mid to late summer produced the greatest increase in female strobili production; the late-July treatment was deemed to be best, tripling strobili yield.

This paper reports the results of a nitrogen fertilization timing study in a young second generation loblolly pine seed orchard.

Materials and Methods

The study was established in a 7-year-old second generation loblolly pine seed orchard in Oak Park, GA. Ammonium nitrate was applied at the rate of 400 pounds per acre in 1992, 1993, and 1994 at four different dates: April 27, June 8, July 27, and September 10. Nineteen clones with two ramets per clone received fertilization at each application time. The experimental design was a randomized complete block with three replication of each treatment and clone combination. The study area of the orchard received standard operational treatment for insects and turf management. Because the orchard was rogued in 1993, only 277 trees of the original 456 were analyzed in the study.

Complete female strobili counts were made in the spring of 1993, 1994, and 1995. Analysis of variance was performed using PROC GLM and clone mean correlation coefficients determined using PROC REG (SAS Institute, Inc. 1989).

Results and Discussion

Female strobili production per tree increased significantly from 1993 to 1995 (40.4 strobili per tree in 1993 to 230.4 strobili per tree in 1995). As anticipated, clonal differences accounted for a substantial portion of the variation in strobili production (Table 1). Similar large clonal effects in seed orchard fertilization studies were also reported by Schmidtling (1975 and 1995).

Table 1. Sources and percent of variation by year in a loblolly pine seed orchard nitrogen timing study.

	Percent of Variation		
Source	1993	1994	1995
Block (B)	0.8	1.4	3.7
Treatment (T)	0	0	0.9
B x T	0	0.5	0
Clone (C)	54.3	59.0	36.2
B x C	5.9	0	8.1
T x C	11.5	1.0	0
B x T x C	10.1	3.4	0
Within	17.4	34.6	51.2
Total	100	100	100

Another aspect of clonal response to fertilization observed in this study, and one that has been widely noted by seed orchard managers, was the year to year consistency of clonal fecundity. Very high (r=0.83** to r=0.84**) (P≤ 0.001) clone mean correlations were obtained in this study (Fig. 1 and Fig. 2). Clone means were very repeatable from year to year; good flowering clones were always good and vice versa.

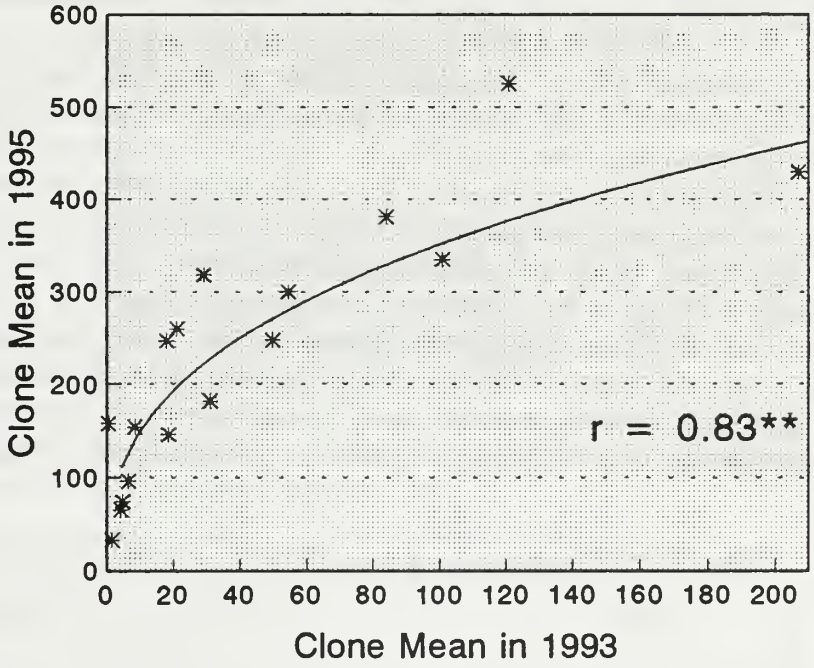

Figure 1. Correlation of clone means for female strobili counts from 1993 (log 1993 counts) with counts from 1995.

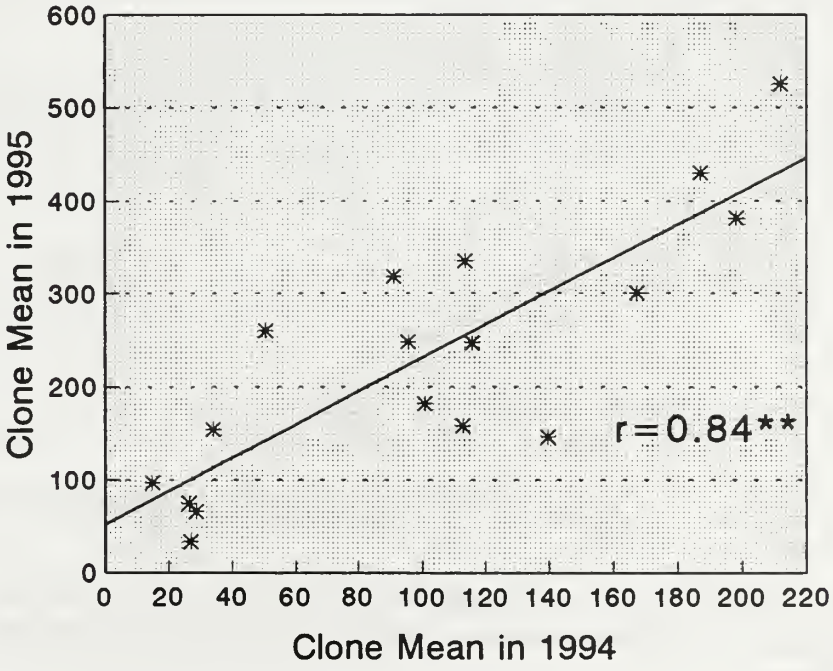

Figure 2. Correlation of clone means for female strobili counts from 1994 and 1995.

In contrast to the large clonal effects, there was virtually no Clone x Treatment interaction. Only 10 percent of the total variation was accounted for by the Clone x Treatment interaction in 1993 and this dropped to 0 percent by the third year of the study (Table 1). A similar lack of clone x timing treatment interaction for female strobili production was also observed by Schmidtling (1975). From an operational seed orchard manager's perspective, this lack of clone by time of fertilizer application greatly simplifies orchard fertilization to promote flowering.

The date of nitrogen application had little influence on female strobili production in 1993 and 1994 (Fig. 3). However, in 1995 the September application date was significantly different ($P \leq 0.05$) from the other application dates (Fig. 3). The lack of a treatment effect in 1993 and 1994 is in direct contrast to the results presented for an older loblolly pine seed orchard by Schmidtling (1975), where there was a marked peak of female strobili production with an August timing of fertilizer application. While the results of the 1995 treatment year do not mimic Schmidtling's results, they too indicate that applications of nitrogenous fertilizers in late summer and early autumn are not as effective as applications made earlier during the growing season (Fig. 3). However, in contrast to Schmidtling's study (1975), the results of this study indicate that there is more latitude in when nitrogen can effectively be applied to stimulate female strobili production. This is evidenced by the lack of treatment differences between the April, June and July application times.

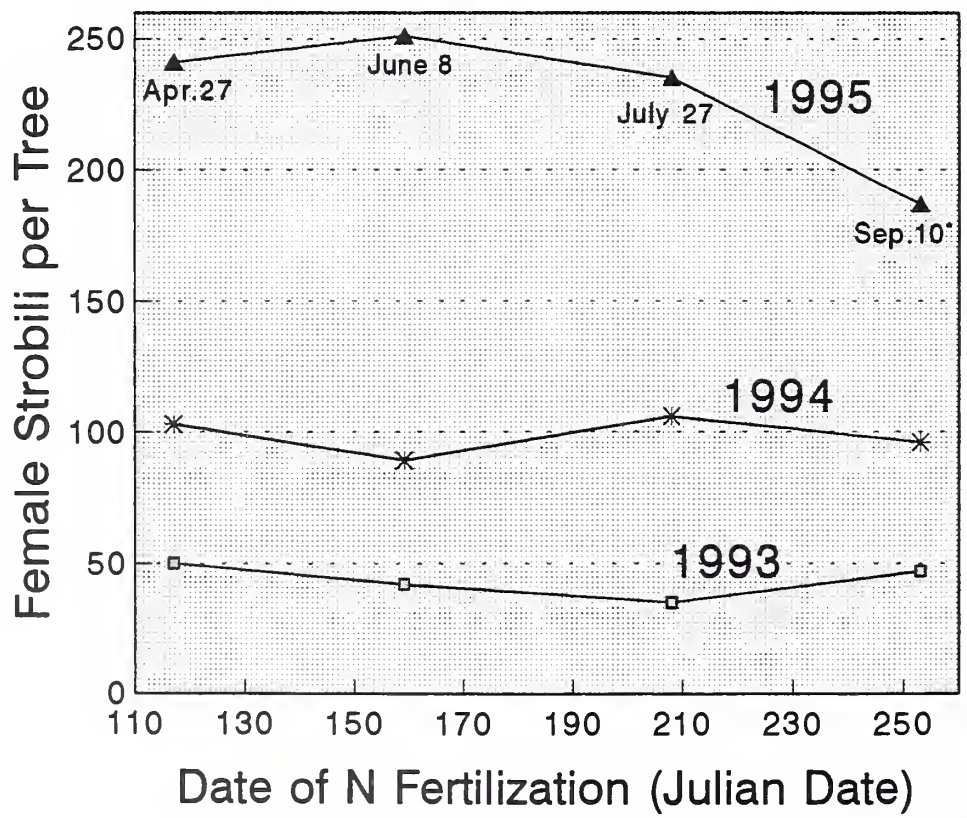

Figure 3. Female strobili counts for different treatment dates for N fertilization in 1993, 1994, and 1995. The only significant treatment difference with any year was the September 10, 1995 application.

Conclusions

This nitrogenous fertilization timing study in a young second generation loblolly pine seed orchard further expands our understanding of seed orchard fertilization practices to promote female strobili formation. There is no evidence that the traditional late-July early-August application of ammonium nitrate is optimal for female strobili stimulation. Female strobili response was similar as long as trees were fertilized during the active growing season. This provides the orchard manager with greater flexibility in scheduling operational nitrogen fertilization. As previous research has also indictaed, very late season application of nitrogenous fertlizers should be avoided.

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PLANT REGENERATION FROM SWEETGUM (*LIQUIDAMBAR STYRACIFLUA*) NODULE CULTURES AND GENETIC TRANSFORMATION BY MICROPROJECTILE BOMBARDMENT

Myoung K. Kim, Harry E. Sommer, Jeffrey F.D. Dean, and Scott A. Merkle

Abstract: Sweetgum (*Liquidambar styraciflua*) nodule cultures were established from seedling hypocotyls and proliferated in liquid Blaydes' medium containing 0.1 mg/l TDZ and 0.01 mg/l 2,4-D. Shoots differentiated from the nodules in liquid Blaydes' medium containing BA (1 mg/l), BA (0.5 mg/l) and NAA (0.01 mg/l), or BA (0.5 mg/l), NAA (0.01 mg/l), and TDZ (0.05 mg/l) in the light. Differentiating shoots required a period of dark treatment for further development on solid medium containing 1 mg/l BA. Elongated shoots were harvested and the cut ends were soaked in 10 mg/l IBA solution prior to planting in potting mix for ex vitro rooting. Roots differentiated and leaves expanded in two weeks. Sweetgum nodules were stably transformed by microprojectile bombardment using a 7.4 kb plasmid, pTRA 140, harboring CaMV 35S-HPH and CaMV 35S-GUS. Evidence that nodules growing in the presence of hygromycin B were stably transformed was provided by PCR analysis and ß-glucuronidase activity. Sweetgum shoots differentiated in liquid medium in the presence of hygromycin B. Shoots transferred to solid medium lacking hygromycin-B elongated and displayed ß-glucuronidase activity in their expanding leaves and stems. PCR analysis confirmed the presence of GUS gene in shoots. Transgenic shoots initiated roots and showed leaf expansion two weeks following planting in potting mix.

Key words: Genetic transformation, nodule culture, microprojectile bombardment, sweetgum, *Liquidambar styraciflua.*

INTRODUCTION

Liquidambar styraciflua L. (sweetgum) is a major hardwood species of the southern United States which is gaining in commercial importance. Incorporation of foreign DNA into sweetgum cells and the subsequent regeneration of transformed plants hold promise for overcoming major obstacles in the conventional genetic improvement of woody perennials, e.g., the transfer of specific genes from other taxa, or modified expression of specific native genes.

Recently, leaf tissue derived from aseptic shoot tip cultures (Sullivan and Lagrimini,1993) and young leaf explants (Chen and Stomp,1992) were transformed through *Agrobacterium*-mediated gene transfer, and transgenic sweetgum trees were regenerated via adventitious shoot induction. *Agrobacterium*-mediated transformation has been pursued in this species due to the immediate initiation of cell division upon wounding and the susceptibility of the species to the bacterium (Stomp, 1991). However, complex host-parasite interactions (Sellmer and McCown, 1989), unanticipated variation (even within a species and clone) in sensitivity to infection (Potrykus, 1990; Binns, 1990; Bergmann and Stomp, 1992, 1994), and low suitability for use with established embryogenic cultures (Merkle et al., 1997) or nodule suspension culture systems of sweetgum could limit the use of the *Agrobacterium*-mediated gene transfer.

On the other hand, biolistic transformation, which involves the acceleration of small metal particles coated with DNA to deliver DNA fragments into plant cells, has been shown to be applicable to a wide range of plant species including forest tree species such as hybrid *Populus* (McCown et al., 1991), yellow-poplar (*Liriodendron tulipifera*) (Wilde et al., 1992; Merkle and Sommer, 1986), and white spruce (*Picea glauca*)(Ellis et al., 1993). The biolistic method has been well integrated

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with embryogenic cultures (Wilde et al., 1992; Ellis et al., 1993) and nodule cultures (McCown et al., 1991), giving the potential to produce transgenic trees on a large scale (Bonga and Von Aderkas, 1992).

The goal of the current study was to establish nodule cultures and to integrate genetic transformation by particle bombardment into this regeneration system for sweetgum, as a potential alternative to the embryogenic culture system. Genetic transformation by particle-bombardment has not yet been demonstrated with sweetgum.

MATERIALS AND METHODS

Nodule culture initiation and maintenance. Sweetgum (*Liquidambar styraciflua*) seeds from a bulked seedlot were surface-sterilized and germinated in vitro on a modified Risser and White's (1964) medium (Sommer and Brown, 1980). Hypocotyl segments were collected when the seedlings were 2 weeks old and cultured on a modified Blaydes' (Witham et al. 1971) medium supplemented with 1 mg/l 2,4-D. Cultures were maintained under a 16-h photoperiod (100 $\mu mol/m^2/s$) provided by cool-white fluorescent lamps at 25 ± 2°C.

Green nodules proliferating along with callus from the hypocotyl cultures were collected and cultured in liquid Blaydes' medium containing the following combinations of TDZ and 2,4-D: TDZ alone at 0.001, 0.01, 0.1, 0.5, or 1 mg/l, 2,4-D alone at 0.01, 0.1, 1, or 10 mg/l, or factorial combinations of TDZ at 0.01, 0.1, or 1 mg/l with 2,4-D at 0.001, 0.01, 0.1, or 1 mg/l. After 6 weeks, nodule cultures were evaluated for response to the different plant growth regulator (PGR) treatments. Based on these results, for subsequent culture, green nodules were collected and proliferated in liquid Blaydes' medium containing 0.1 mg/l TDZ and 0.1 or 0.01 mg/l 2,4-D. At 3 week intervals, small (less than 3 mm in diameter), green, nodular aggregates were collected and subcultured at an inoculum density of 1 ml of settled nodule volume (SNV) per 40 ml of liquid medium in 125 ml Erlenmeyer flasks. Cultures were maintained on an orbital shaker at 100 rpm under the same conditions used for nodule initiation.

Shoot regeneration and plantlet production. Nodules were induced to produce adventitious shoots in liquid medium containing one of the following combinations of growth regulators: TDZ (0.2 mg/l) ; BA (0.5 mg/l) and NAA (0.01 mg/l); TDZ (0.5 mg/l), BA (0.5 mg/l) and NAA (0.01 mg/l). In a preliminary experiment, shoots proliferated on solid medium in the light first turned red and then darkened and died when cultures were maintained in the light. When maintained in the dark, most shoots remained alive. Therefore, nodules showing shoot differentiation in liquid medium were transferred to solid medium containing 1 mg/l BA and subjected to 0, 2, 4, or 6 weeks of dark treatment. Cultures were transferred to fresh medium every 6 weeks. Shoots longer than 1 mm were counted at 10 weeks of culture. Data were analyzed by GLM (SAS/STAT User's Guide, 1988). Elongated shoots greater than 2 cm were harvested and the cut ends were soaked in 10 mg/l IBA solution for 10 minutes prior to planting in potting mix for ex vitro rooting (Kim et al., 1997).

Transformation and analysis of transgenic nodule cultures. The plasmid pTRA 140, harboring CaMV 35S-HPH and CaMV 35S-GUS (provided by Dr. Murai, department of plant pathology and crop physiology, Louisiana State University) was used for microprojectile bombardment. Plasmid DNA was loaded onto gold particles and bombarded onto nodule culture cells following the DuPont DNA preparation protocol (Fraley et al., 1984). Newly proliferating nodules were fractionated through 20 mesh (1.2 mm) and 40 mesh (0.38 mm) screens 2 weeks following subculture and maintained in fresh liquid medium supplemented with 0.1 mg/l TDZ and 0.01 mg/l 2,4-D or 0.1 mg/l 2,4-D for 3 days prior to bombardment. In preparation for bombardment, harvested nodules were placed on filter paper supports, blotted thoroughly with sterile blotting

paper and transferred to empty Petri dishes. One ml of SNV was used per plate. Following bombardment, nodules were transferred to non-selective semisolid medium with 0.1 mg/l TDZ and 0.01 mg/l 2,4-D, and incubated in the dark. After 2 days, nodules subsampled for histochemical GUS assay were stained with 5-bromo-3-chloro-3-indolyl-ß-D-glucuronic acid solution (Jefferson et al., 1987). Cells were incubated at 25°C overnight and the number of blue spots was counted using a dissecting microscope. The remaining nodules were maintained for one week on non-selective medium and then transferred to selection medium containing the same PGRs, but with 5 mg/l hygromycin B. The concentration of hygromycin B was raised to 10 mg/l at the third week of selection and to 15 mg/l at the eighth week of selection.

To provide evidence of stable transformation, nodules that had been under selection for 3 months were subjected to polmerase chain reaction (PCR) or histochemical GUS assay (Jefferson et al., 1987). For PCR, DNA was extracted from about 25 mg of fresh nodule cells by following the procedure of Stewart et al. (1993). To detect the relevant portion of the introduced DNA, a 1200 bp sequence was amplified from a non-intron GUS gene by using the primers designed by Jefferson et al. (1986). Nodules (and later, shoot primordia, stems and expanding leaves) were assayed for GUS activity using the same histochemical assay used for transient expression. From the GUS positive transgenic shoots, DNA was extracted and used for PCR analysis.

Those nodule lines displaying evidence of stable transformation by GUS activity or PCR analysis were induced to produce adventitious shoots in the same liquid medium used for production of shoots from non-transgenic cultures, but containing 5 mg/l hygromycin B. When shoot clumps were transferred onto solid medium for elongation, hygromycin B was omitted.Elongated shoots were planted in potting mix for ex vitro rooting following the same procedure used for non-transgenic shoots.

RESULTS AND DISCUSSION

Nodule culture initiation and maintenance. Nodules, collected from callus masses from one to two months following hypocotyl culture initiation, were dense cell masses that were independent and formed spherical, cohesive units, fitting the description of nodules given by McCown et al. (1988). Suspension cultures initiated from these nodules were evaluated for the impact of the different PGR combinations on morphology, proliferation, and homogeneity after 6 weeks. Optimal proliferation and homogeneity of nodules were obtained with 0.1 mg/l TDZ and 0.01 or 0.1 mg/l 2,4-D. At levels of 2,4-D above 1 mg/l, with or without TDZ, cultures did not proliferate as green nodules. With TDZ alone most nodules turned green within a few weeks and some showed shoot differentiation. At TDZ concentrations above 1 mg/l, some nodule cultures turned dark and ceased further proliferation.

With levels of TDZ above 0.5 mg/l and 2,4-D above 1 mg/l, nodule size increased, resulting in highly heterogeneous suspension cultures. Treatment with 0.1 mg/l TDZ and 0.1 mg/l or 0.01 mg/l 2,4-D gave rise to three types of cell masses: (1) Green nodules, (2) white nodules, and (3) unorganized cell masses. With 0.1 mg/l TDZ and 0.01 mg/l 2,4-D, most of the nodules remained green and compact 3 weeks following subsequent 3-week subcultures (Type I). On the other hand, cultures maintained with 0.1 mg/l TDZ and 0.1 mg/l 2,4-D gave rise to all three types of cell masses mentioned above, but with only a small amount of green nodular cell masses at the end of 3 weeks of culture (Type II). Both types produced white or yellow daughter nodules within a week following subculture, but most of these turned green at the end of 3 weeks in Type I nodule cultures. Type II cultures lost their potential for adventitious shoot production within 6 months, but Type I cultures have maintained their potential for adventitious shoot differentiation for over 1 year. In addition, competent nodule cultures could be renewed by transferring the shoot differentiating nodules from BA-containing liquid medium (see below) to nodule proliferation medium containing 0.1 mg/l TDZ and 0.01 mg/l 2,4-D.

Shoot regeneration and plantlet production. In a preliminary study, shoot initiation from nodule cultures was initially observed following 6 weeks of culture in liquid medium containing one of the following combinations of growth regulators: TDZ (0.2 mg/l); BA (1 mg/l); BA (0.5 mg/l) and NAA (0.01 mg/l); TDZ (0.5 mg/l), BA (0.5 mg/l) and NAA (0.01 mg/l). However, shoot initiation was not observed on solid medium. In the current study, however, when nodules showing shoot differentiation were maintained in liquid medium, most of shoot primordia turned red and eventually lost their morphology with severe vitrification as described by Debergh et al. (1992). Therefore, they were transferred to solid medium to avoid further vitrification. Even on solid medium, most shoot primordia turned red with subsequent darkening and died in the light. When they were maintained in the dark, most shoot primordia retained their morphology, and new shoots emerged from the mass. This latter observation prompted us to test the effects of dark treatment on shoot formation and elongation on solid medium. A clear difference was shown in shoot formation between the control (no dark treatment) and the dark treatments (Fig. 2). Four weeks of dark treatment gave the highest shoot formation of 11 shoots per nodule cluster. When the differentiated shoots were exposed to light following dark treatment, they developed normally and leaves greened and expanded on solid medium containing 1mg/l BA. Dark treatment was apparently necessary to allow the vitrified shoots to recover physiologically for continued development on solid medium. All shoots transferred to potting mix rooted and leaves expanded two weeks following planting in potting mix(data not shown).

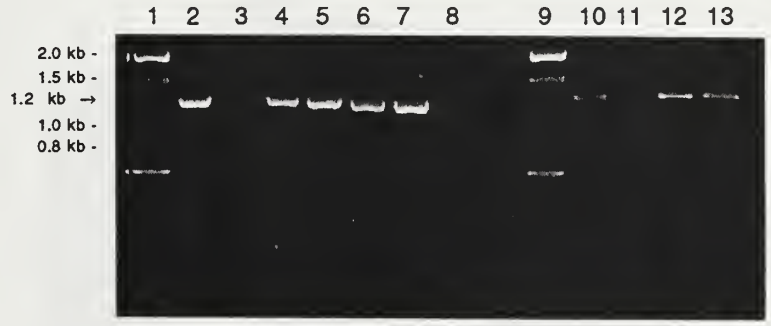

Figure 1. PCR analysis of the transgenic nodule cultures and the transgenic shoots for the integration of GUS gene. *Lanes 5-8* contained genomic DNA from GUS (+) independent Type II nodule cultures; *Lane 8* from GUS (-) Type II nodule cultures; *Lane 12* from GUS (+) Type I nodule cultures; *Lane 13* from GUS (+) shoots; *lanes 1* and *9* contained 100-bp DNA ladder; *lanes 3* and *11* represent the negative control of non-transformed sweetgum nodule cultures; *lanes* 2 and *10* contained the pTRA 140 plasmid of positive control containing GUS gene.

Transformation and analysis of transgenic nodule cultures. The highest levels of transient GUS expression observed were approximately 2,000 and 2,400 blue spots per 1 ml of settled nodule volume (SNV) from Type I and Type II nodules, respectively. Zero to three hygromycin-resistant nodules were recovered per plate, for a total of thirteen lines of hygromycin resistant nodules. Nine of these lines were Type II nodules. Of the five lines tested for the insert using PCR, four showed 1200 bp bands corresponding to the 1200 bp band from the positive control pTRA 140 plasmid (Fig. 1). Among the nine hygromycin-resistant lines, one showed only root differentiation in the presence of hygromycin in the medium containing 0.1 mg/l TDZ and 0.01 mg/l 2,4-D, and was GUS. On the other hand, the four hygromycin resistant lines derived from Type I nodules were all GUS positive. Of the four lines, one showed the typical morphology of Type I nodules and has maintained shoot differentiation in the liquid selection medium used for shoot induction. The other three lines resembled Type II nodules and did not produce shoot. As was the case for

non-transformed nodules, four weeks of dark treatment was optimal for shoot production, with an average of 14 shoots produced per nodule cluster (Fig. 2). At the time of rooting, most of the transgenic shoots, grown without hygromycin, were GUS positive in the stems and expanding leaves. However, some of the shoots were GUS negative even though they were derived from GUS positive, hygromycin resistant nodules. GUS positive transgenic shoots began to root *ex vitro* and leaves expanded two weeks following planting in potting mix. Five out of fifteen GUS positive transgenic shoots showed root initiation and leaf expansion within four weeks.

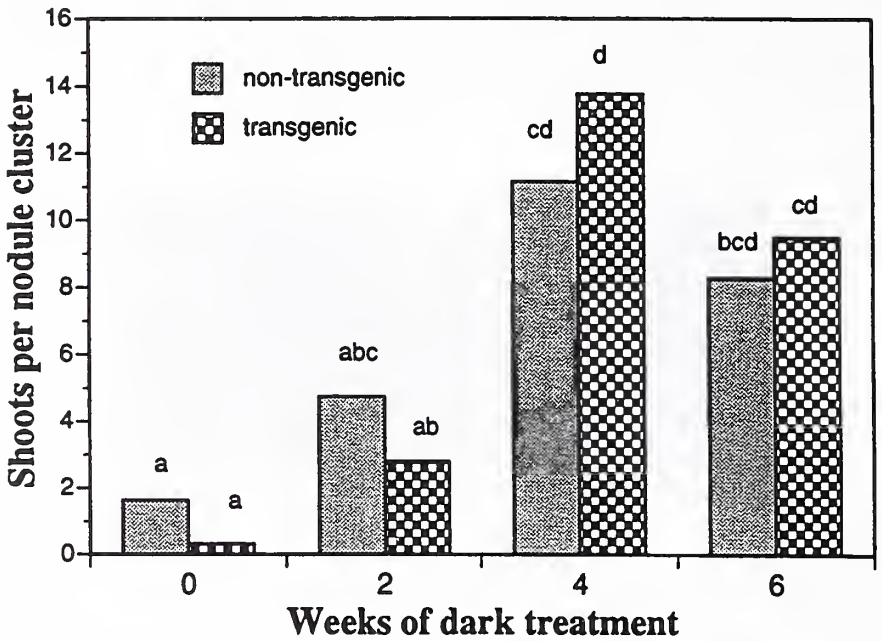

Figure 2. Effect of dark treatment on shoot production on solid medium from non-transgenic and transgenic nodules: Shoots greater than 1 mm were counted from 10 to 87 replicates at 10 weeks of culture. Means with the same letters are not significantly different at the 0.01 level using Duncan's multiple range test.

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RAPD MAPPING OF GENOMIC REGIONS INFLUENCING EARLY HEIGHT GROWTH IN LONGLEAF PINE × SLASH PINE F_1 HYBRIDS

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Abstract: Despite many desirable qualities, longleaf pine has found limited use in artificial regeneration programs because of its often poor survival rate and extended phase (2-20+ yrs) of reduced height growth referred to as the grass stage. Interspecific hybrids of longleaf pine have shown promise for addressing the problem of delayed height growth. Considerable variation in height growth has been observed in various longleaf pine x slash pine hybrid families suggesting that an interspecific backcross breeding approach might be successfully employed to eliminate the grass stage in longleaf pine. Automation of the random amplified polymorphic DNA (RAPD) technique has now made it possible to quickly identify large numbers of markers that can be used to construct genetic linkage maps, tag genes of interest, and accelerate the introgression of genes in backcross breeding programs. The objective of this study was to use RAPD marker maps for longleaf pine and slash pine to obtain preliminary information on the genomic location of regions influencing early height growth (EHG). Seventy-two F_1 trees generated from an interspecific cross between longleaf pine and slash pine were used. Genetic maps were constructed for each parent with markers segregating in a 1:1 Mendelian ratio. Both linked and unlinked markers were used to search for genomic regions influencing EHG. Total height was measured six times over four years (3, 5, 9, 21, 33, and 45 months). Several regions controlling part of the variation for EHG were identified by single marker regression analyses. Based on age by age analyses, some temporal stability of quantitative trait loci (QTL) expression was observed. One genomic region in longleaf pine was significantly associated with EHG at all six measurement dates. These preliminary results are very encouraging for the application of markers to identify genomic regions influencing EHG. They should also prove to be extremely powerful in the backcross populations where we will be using larger progeny sizes and expect more genes to be segregating.

Keywords: *Pinus palustris* Mill., *Pinus elliottii* Engelm., interspecific hybrids, grass stage, RAPD markers.

INTRODUCTION

Despite many desirable qualities, longleaf pine (*Pinus palustris* Mill.) has found limited use in artificial regeneration programs due to complications associated with an extended phase of juvenile development referred to as the grass stage (Schmidtling and White 1989). The grass stage greatly increases the opportunity for brown-spot needle blight infection caused by the fungus *Scirrhia acicola* (Dearn.) [Siggers 1944]. This disease can greatly prolong the grass stage

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and, if severe enough, can kill seedlings. Increased mortality (compared to the other southern pines) and the unpredictability of the duration of the grass stage make planting longleaf pine a risky investment under intensive management systems.

Inter-specific hybrids of longleaf pine have shown promise for addressing the problem of delayed height growth. Intermediate early height growth has been observed in various families of longleaf pine crossed to either loblolly pine or slash pine (Brown 1964; Derr 1966; 1969). Analysis of various F_2 and BC_1 families suggest that as few as 5-10 loci may control early height growth (EHG) [Brown 1964; C. D. Nelson unpublished data]. Although current estimates of the number of loci influencing EHG are based on only a few hybrid families, they do suggest that the grass stage character is a quantitative trait controlled by a finite number of genes (oligogenic vs. polygenic).

Recent advances in DNA-based marker technology have made it possible to conduct efficient genetic mapping and QTL searching experiments. Automated approaches afforded by the RAPD technique are offering enormous benefits in terms of time and labor (Grattapaglia et al. 1992; Nelson et al. 1994). Low- to medium-density RAPD maps have recently been published for several conifer species such as white spruce (Tulsieram et al. 1992), slash pine (Nelson et al. 1993; Kubisiak et al. 1995), longleaf pine (Nelson et al. 1994; Kubisiak et al. 1995), Norway spruce (Binelli and Bucci 1994), and maritime pine (Plomion et al. 1995). The RAPD technique is well-suited to genetic mapping in highly heterozygous outcrossed species.

In the present study, we used RAPD marker linkage maps constructed for the parents of a longleaf pine × slash pine F_1 family (Kubisiak et al. 1995) to perform preliminary searches for genomic regions influencing EHG. We present the results of these searches and relate the findings to our backcross breeding program designed to introgress genomic regions promoting EHG from slash pine into longleaf pine.

MATERIALS AND METHODS

Mapping Population. Seventy-two progeny from an interspecific cross between longleaf pine 3-356 (♀) × slash pine H-28 (♂) were employed for genomic mapping and QTL searching. Seeds were germinated and grown in containers in a greenhouse at the Southern Institute of Forest Genetics previously located in Gulfport, MS. At four months of age, the seedlings were transplanted to a nursery bed at the Harrison Experimental Forest in Saucier, MS and grown for another five months. During this period all seedlings were protected from damping-off and brown spot needle blight and given uniform growing conditions. At nine months, the seedlings were out-planted to a field site on the forest and allowed to grow under natural conditions.

Growth Variables. The growth variables measured included; height in containers at three months, height in the nursery bed at five months and nine months, and height in the field at 21 months, 33 months, and 45 months.

DNA Extraction and RAPD Amplification. DNA was isolated and genetic polymorphisms were amplified using the polymerase chain reaction as described in Kubisiak et al. (1995). Segregating

RAPD markers were identified by the manufacturer primer code corresponding to the 10-mer primer responsible for their amplification, followed by a subscript four digit number indicating the approximate band size in base pairs. Those cases in which the presence or absence of bands was unclear, were recorded as missing data.

Segregation Analysis. The marker data were entered into the computer package MAPMAKER/EXP (version 3.0) and analyzed using a modified backcross format (Nelson et al. 1993). The mapping strategy used was similar to that suggested in Lincoln et al. (1992). Linkage groups were assigned three letter names. The first two letters designate species (Pp = *Pinus palustris*, Pe = *Pinus elliottii*), and the third designates individual linkage groups. The longleaf pine linkage groups were assigned names according to designations proposed in Nelson et al. (1994).

Molecular Evaluation of Growth Data. The degree of association between the marker loci and the EHG data was investigated by employing single-locus ANOVA models in which the individual marker-genotypes were used as class variables (Keim et al. 1990). An association between a marker and the height growth data was considered significant if the probability of observing a F-value as large as or larger than the observed value was ≤ 0.05. This threshold was chosen in an attempt to lower the type II error rate (Jansen 1994). The association of any marker found to be significantly associated with EHG for at least one age was further examined across all ages. Temporal examination of association as well as additional linkage information provided by the map were used as criteria for assessing whether a genomic region is associated with EHG.

RESULTS

Longleaf Pine and Slash Pine Linkage Maps. A total of 132 and 101 loci were used to construct recombinational linkage maps for longleaf pine and slash pine, respectively. A total of 122 (92.4%) of the longleaf pine loci were found to be linked at LOD$\geq$4.0. These loci mapped to 18 groups and three pairs spanning a total genetic distance of 1367.5 Haldane centiMorgans (cM). The genetic distance between markers ranged from 0.0 cM to 35.3 cM, with an average spacing of 11.2 cM. Of the 101 slash pine loci, 91 (90.1%) were found to be linked at LOD$\geq$4.0. These loci mapped to 13 groups and six linked pairs spanning a total genetic distance of 952.9 Haldane cM. The genetic distance between markers ranged from 0.0 cM to 38.3 cM, with an average spacing of 10.5 cM.

Genomic Regions Influencing EHG. The distribution of early height growth at each age was found to approximate a normal distribution (data not shown). The mean and standard deviation of height growth at each age is presented for the F_1 family, as well as for open-pollinated families from each parent (Table 1). The mean height growth for the F_1 family was roughly intermediate to the open-pollinated families at all ages, but appears to harbor considerable variation suggesting that some genetic loci influencing EHG might be segregating.

Table 1. Mean and standard deviation of height growth at six different ages for three families: open-pollinated family from longleaf pine 3-356; open-pollinated family from slash pine H-28; and a full-sib family 3-356 x H-28. Shown are; family (Family), height growth measure (Variable), number of progenies (N), mean height growth in centimeters (Mean), standard deviation about the mean (Std. Dev.).

Family	Variable[1]	N	Mean	Std. Dev.
3-356 OP	HT3	133	0.303	0.369
3-356 x H-28		72	7.340	1.523
H-28 OP		82	14.183	2.177
3-356 OP	HT5	132	1.545	0.695
3-356 x H-28		72	7.625	1.819
H-28 OP		82	25.963	3.766
3-356 OP	HT9	133	3.481	1.535
3-356 x H-28		72	24.153	7.380
H-28 OP		82	59.268	13.075
3-356 OP	HT21	119	7.134	2.724
3-356 x H-28		70	36.614	12.210
H-28 OP		82	80.524	18.363
3-356 OP	HT33	110	37.764	45.788
3-356 x H-28		57	151.386	69.345
H-28 OP		82	221.489	42.155
3-356 OP	HT45	97	74.536	90.224
3-356 x H-28		54	253.315	93.832
H-28 OP		81	339.160	38.622

[1]HT3=height at three months; HT5=height at five months; HT9=height at nine months; HT21= height at 21 months; HT33= height at 33 months; HT45=height at 45 months.

A total of 24 markers located on eight different longleaf pine linkage groups were found to be significantly associated (Prob.>F≤0.05) with height growth for at least one age based on single-marker ANOVAs (Table 2). Sixteen of these markers were associated with EHG at only one age and had no apparent pattern of temporal expression. Six markers located on linkage group PpC were significantly associated with EHG at more than one age. Marker A09_1300 was significantly associated with EHG at all six ages. At marker A09_1300, the band-present allele was associated with reduced height growth. By 45 months of age, individuals possessing one copy of this allele were an average of 57.5 cm (0.61 phenotypic standard deviations) shorter than individuals possessing the alternate null allele (Table 3). Two markers (102_0550 and 297_0950) located on linkage group PpI were associated with EHG only at 45 months, however, both clearly exhibit an increasing trend in significance through time suggesting that a region on linkage group PpI may influence EHG. At marker 102_0550, the band-present allele from longleaf pine was associated with reduced height growth. By 45 months of age, individuals possessing one copy of this allele were an average of 56.7 cm (0.60 phenotypic standard deviations) shorter than individuals possessing the alternate null allele (Table 3).

Table 2. Longleaf pine markers significantly associated with early height growth (Prob.>F≤0.05) for at least one date based on single marker analysis of variance. Shown are; marker (Marker), linkage group (LG), marker order in the linkage group (MO), height at three months (HT3), height at five months (HT5), height at nine months (HT9), height at 21 months (HT21), height at 33 months (HT33), height at 45 months (HT45).

			Prob.>F					
Marker	LG[1]	MO[2]	HT3	HT5	HT9	HT21	HT33	HT45
322_0525	PpB	NA	0.161	0.591	0.166	0.277	**0.048**	0.186
169_1500	PpC	1	0.083	**0.019**	0.568	0.645	0.411	0.649
608_0600	PpC	2	0.268	**0.042**	0.976	0.681	0.164	0.108
111_0850	PpC	3	**0.020**	0.391	0.054	0.086	0.714	0.778
111_0900	PpC	4	**0.020**	0.391	0.054	0.086	0.714	0.778
543_0725	PpC	5	**0.003**	**0.023**	**0.018**	0.053	0.436	0.642
A16_1100	PpC	6	**0.007**	**0.010**	**0.024**	0.051	0.145	0.215
333_0650	PpC	7	**0.003**	**0.008**	**0.011**	**0.028**	0.259	0.222
119_0525	PpC	8	**0.046**	**0.013**	0.108	**0.044**	0.071	0.057
A09_1300	PpC	9	**0.041**	**0.008**	**0.049**	**0.019**	**0.039**	**0.025**
171_1500	PpC	10	0.124	0.059	0.077	**0.012**	0.062	**0.043**
A08_0900	PpC	11	0.138	0.140	0.151	**0.022**	0.173	0.211
159_0550	PpD	1	**0.036**	0.883	0.959	0.943	0.322	0.454
544_1700	PpD	2	**0.019**	0.912	0.431	0.772	0.652	0.916
J08_0700	PpD	3	**0.048**	0.789	0.453	0.834	0.467	0.959
W19_0600	PpE	NA	0.755	0.325	0.293	0.056	0.082	**0.025**
257_1000	PpE	NA	**0.019**	0.149	0.205	0.084	0.498	0.769
A11_1400	PpG	NA	0.278	0.811	**0.021**	0.095	0.779	0.653
499_0800	PpH	NA	**0.025**	0.112	0.780	0.863	0.707	0.941
X04_1000	PpI	1	0.511	0.369	**0.010**	0.134	0.170	0.217
102_0550	PpI	2	0.755	0.325	0.293	0.056	0.082	**0.025**
297_0950	PpI	3	0.981	0.408	0.270	0.054	0.108	**0.040**
123_0525	PpN1	NA	0.752	0.161	0.093	**0.035**	0.208	0.340
360_1100	UL	NA	**0.036**	0.206	0.849	0.533	0.114	0.343

[1]LG=linkage group; UL=unlinked
[2]MO=marker order; NA=not applicable

Table 3. Mean height growth at 45 months of age for individuals harboring alternative marker genotypes. Shown are; species, marker, marker genotype (Genotype), number of individuals with particular marker genotype (N), mean height at 45 months of age for individuals with particular marker genotype (HT45), standard deviation (Std. Dev.).

Species	Marker	Genotype[1]	N	HT45 (cm)	Std. Dev.
Longleaf pine	A09_1300	a	32	276.75	89.48
		A	22	219.23	91.35
	102_0550	b	29	279.55	79.03
		B	25	222.88	101.78
Slash pine	452_1700	c	25	285.76	80.24
		C	23	232.52	101.72
	X04_0550	D	25	290.08	60.38
		d	29	221.62	106.27

[1]a = null allele at marker A09_1300 associated with increased height growth
b = null allele at marker 102_0550 associated with increased height growth
c = null allele at marker 452_1700 associated with increased height growth
D = band-present allele at marker X04_0550 associated with increased height growth

A total of 15 markers located on six different slash pine linkage groups were found to be significantly associated (Prob.>F≤0.05) with height growth for at least one age based on single-marker ANOVAs (Table 4). Ten of these markers were associated with EHG at only one age and had no apparent pattern of temporal expression. Two markers (E02_0700 and 268_1200) located on linkage group PeG were significantly associated with EHG at 3 and 5 months, however, there effect diminished once the trees were put into the field. An unlinked marker (638_0330) was significantly associated with EHG at 9 and 21 months, however, no association was noted at 33 or 45 months. Two markers 452_1700 and X04_0550 showed trends of an increasing association through time. Marker 452_1700 is one member of a linked pair. At this marker, the null allele was associated with increased height growth. By 45 months of age, individuals possessing one copy of this allele were an average of 53.24 cm (0.57 phenotypic standard deviations) taller than individuals possessing the alternate band-present allele (Table 3). Marker X04_0550 is an unlinked marker. The band-present allele at this locus was associated with increased height growth. By 45 months of age, individuals possessing one band-present allele were an average of 68.46 cm (0.73 phenotypic standard deviations) taller than individuals possessing one copy of the alternate null allele (Table 3).

Table 4. Slash pine markers significantly associated with early height growth (Prob.>F≤0.05) for at least one date based on single marker analysis of variance. Shown are; linkage group (LG), marker order (MO), height at three months (HT3), height at five months (HT5), height at nine months (HT9), height at 21 months (HT21), height at 33 months (HT33), height at 45 months (HT45).

			Prob.>F					
Marker	LG[1]	MO[2]	HT3	HT5	HT9	HT21	HT33	HT45
660_0400	PeB	NA	0.986	0.974	0.279	0.053	**0.016**	0.053
698_2200	PeC	NA	**0.025**	0.119	0.404	0.767	0.547	0.286
631_0800	PeE	NA	0.202	**0.024**	0.543	0.260	0.314	0.962
E02_0700	PeG	NA	**0.048**	**0.003**	0.764	0.156	0.597	0.906
268_1200	PeG	NA	**0.012**	**0.002**	0.855	0.502	0.888	0.590
G09_0750	PeJ	NA	**0.009**	0.421	0.202	0.146	0.646	0.524
460_0600	PeN	NA	0.224	0.366	0.128	0.486	0.230	**0.031**
485_1100	LP	NA	0.222	0.393	0.142	0.366	0.263	**0.032**
452_1700	LP	NA	0.943	0.568	0.297	**0.019**	**0.014**	**0.049**
299_1300	LP	NA	0.793	0.936	0.673	0.754	0.342	**0.026**
X04_0550	UL	NA	0.365	0.391	**0.014**	**0.003**	**0.008**	**0.006**
G09_0500	UL	NA	0.129	**0.040**	0.454	0.277	0.066	0.103
B08_2200	UL	NA	**0.023**	0.145	0.489	0.924	0.617	0.456
638_0330	UL	NA	0.187	0.146	**0.021**	**0.004**	0.295	0.634
314_0950	UL	NA	0.486	0.095	0.076	**0.047**	0.150	0.183

[1]LG=linkage group; LP=linked pair; UL=unlinked
[2]MO=marker order; NA=not applicable

For the four marker loci A09_1300, 102_0550, 452_1700 and X04_0550 the mean and standard deviation of height growth for individuals possessing zero, one, two, three, or four positive-effect height growth alleles are displayed in Table 5. Individuals possessing four positive effect height growth alleles were an average of 152.3 cm (1.62 phenotypic standard deviations) taller than individuals harboring no positive-effect alleles. At 45 months of age, four of the eight shortest trees and four of the eight tallest trees could be identified by alleles at these four loci (data not shown). The other four individuals in each tail of the distribution could be

identified by alleles at three of these loci, and were most likely recombinants at the fourth locus.

Table 5. Mean height growth at 45 months of age for individuals harboring different numbers of putative alleles for early height growth (EHG) based on molecular marker genotypes: Shown are; marker genotype (Genotype), number of positive-effect EHG alleles (NPA); number of individuals with particular marker genotype (N), mean height at 45 months of age for individuals with particular marker genotype (HT45), standard deviation (Std. Dev.).

Genotype	NPA	N	HT45 (cm)	Std. Dev.
a,b,c,D	4	8	317.63	52.47
a,b,c,d	3	11	297.45	52.81
a,b,C,D				
a,B,c,D				
A,b,c,D				
a,b,C,d	2	16	266.62	95.14
a,B,c,d				
A,b,c,d				
a,B,C,D				
A,b,C,D				
A,B,c,D				
a,B,C,d	1	7	203.00	101.69
A,B,C,D				
A,b,C,d				
A,B,c,d				
A,B,C,d	0	6	165.33	101.91

[1]a = null allele at marker A09_1300 associated with increased height growth
b = null allele at marker 102_0550 associated with increased height growth
c = null allele at marker 452_1700 associated with increased height growth
D = band-present allele at marker X04_0550 associated with increased height growth

DISCUSSION

In the present study, we utilized recombinational linkage maps constructed for the parents of a longleaf pine x slash pine F_1 cross to search for genomic regions influencing (EHG). Using the molecular marker and field data we identified two regions on the longleaf pine genome and two regions on the slash pine genome that putatively influence EHG. The fact that RAPD markers segregating in a 1:1 Mendelian fashion provide linkage information for only a single parent (Grattapaglia et al. 1992) precluded our ability to determine whether the genomic regions identified in each parental species are located on homologous chromosomes.

Another drawback associated with the dominant nature of RAPD markers is when the null allele is associated with the allele of interest. For example, the null allele for slash pine marker 452_1700 was associated with increased height growth. If we were to select for the null allele at this locus in our BC_1 populations we might erroneously be selecting for the null allele inherited from longleaf pine, which may or may not be associated with the allele of interest for our trait. Some ways around this potential problem might be to convert these RAPD markers into codominant sequence characterized amplified region markers, or to perform bulked

segregant analysis on these markers to try to saturate these regions with other types of codominant markers such as microsatellites or restriction fragment length polymorphisms. Codominant markers might allow us to select for parent-specific alleles and could potentially bypass the problems associated with the null RAPD allele.

It should be stated that these results are only preliminary. The power of these analyses was limited by the rather small number of progeny available. These results have, however, brought to our attention several regions in longleaf pine and slash pine that should be given further attention in our BC_1 populations.

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FUSIFORM RUST RESISTANCE OF CENTRAL AMERICAN AND MEXICAN PINE SPECIES COMPARED WITH LOBLOLLY AND SLASH PINES

C. C. Lambeth[1], W. S. Dvorak[2], and C. H. Young[3]

Abstract:--Seedlings of five Central American and Mexican pine species and loblolly (*Pinus taeda* L.) and slash (*Pinus elliottii* Engelm. var. *elliottii*) pines were tested for fusiform rust (*Cronartium quercuum* f. sp. *fusiforme*) resistance at the USDA Forest Service Resistance Screening Center to determine the potential value of exotic species for improving rust resistance through hybridization with native species in the southeastern U.S..

The average infection of all species was 76%. *Pinus teocote* Schl. & Cham. was by far the most resistant with an infection rate of only 29% and the next best lot was *Pinus greggii* Engelm. (Valle Verde provenance) with an infection rate of 70%. *Pinus caribaea* Morelet var. *hondurensis* was the most susceptible species with 93% infection. "Resistant" (genetically improved) and "susceptible" slash pine lots showed 71% and 92% infection, respectively, indicating that genetic improvement of native species can result in infection rates as low or lower than that of the more resistant tropical pine species tested.

Pinus teocote is a good candidate for hybridization with native species due to its excellent rust resistance and it may offer potential to improve growth rate as well since it comes from lower latitudes than slash and loblolly. Under the right conditions, pines from more tropical regions will often outgrow temperate pines. It would be necessary to determine the cold adaptability in the southeastern U.S. of any hybrid between local species and tropical pines. The authors discuss the potential of temperate by tropical pine species hybrids.

Key Words: Tropical pines, *Pinus taeda*, *Pinus elliottii*, *Cronartium quercuum* f. sp. *fusiforme*, resistance screening, hybrid.

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INTRODUCTION

The CAMCORE (Central American and Mexican Coniferous Resources) Cooperative has made mother tree collections of tropical and subtropical pine species in Central America and Mexico since its formation in 1980 (Dvorak and Donahue 1992). The commercial value of some of these species was unknown in the beginning but some such as *Pinus tecunumanii* (Schw.) Eguiluz et Perry (Dvorak and Ross 1994), *Pinus chiapensis* L. (Dvorak et al. 1996a) and *Pinus greggii* (Dvorak et al. 1996b) are now being planted commercially or they are being considered for commercial use in the tropics and subtropics. It will become increasingly important to understand the degree of tolerance of these promising species to pests common to pine species in regions of large scale commercial plantations. For example, pitch canker (*Fusarium subglutinans* f. sp. *pini*) has recently been identified on *Pinus radiata* in California (Storer et al. 1994) and on *Pinus patula* in South Africa (Viljoen and Wingfield 1994). Little is known how this potentially serious disease will affect other tropical and subtropical pine species when planted as exotics.

Fusiform rust resistance of many pine species is not well studied either. In areas where the alternate host exists it is of value to know the rust resistance of exotic species in order to determine whether or not rust will be a serious problem. Furthermore, resistance information is needed to determine the potential value of a new species for hybridization with local species.

We were interested in determining the relative rust resistance of some Central American and Mexican pine species that have potential commercial value in the subtropics or in temperate regions as hybrids with local species in the southeastern U.S.. Most of these species will not likely be adapted to the climatic conditions in the southeastern U.S., specifically because they come from lower latitudes that do not experience the extremes in cold temperatures or extreme temperature change found here. Nonetheless, they may have value as hybrids with native pines if the local species can provide the adaptation to the regional climate and if the nonlocal species can convey to the hybrid some complementary characteristic such as faster growth, resistance to disease or desirable wood quality traits.

The objective of the study was to determine the relative rust resistance of seedlings of six Central American and Mexican pine species in comparison with three native species of commercial value in the southeastern U.S..

MATERIALS AND METHODS

Seed from 10 to 14 mother tree seed collections per Central American and Mexican pine species were mixed to form the seedlots used in the study. The species, provenance and some information at the area of origin can be seen in Table 1.

Table 1. Information on the areas of origin of the tropical and subtropical pine species screened for fusiform rust resistance.

PINE SPECIES	PROVENANCE	COUNTRY	ELEV. (m)	LAT.	LONG.	MEAN ANN. TEMP.C^0	ANN. PRECIP. (mm)
caribaea var *hond.*	Alamikamba	Nicaragua	30	13°34′	84°17′	27.3	2610
greggii	San Joaquin	Mexico	2350	20°56′	99°34′	14.0	1100
	Valle Verde	Mexico	1200	21°29′	99°12′	17.0	1400
herrarae	Guajolata	Mexico	2100	23°13′	105°11′	11.4	927
leiophylla	San Isidro	Mexico	2400	23°39′	105°02′	11.4	927
tecunumanii	San Jeronimo	Guatemala	1750	15°03′	90°18′	18.0	964
teocote	Maguayes	Mexico	2400	20°26′	98°30′	13.5	1341

Shortleaf pine (*Pinus echinata* Mill.) and *Pinus herrarae* Mart. did not have sufficient germinants to be included in the study. The local species checks are described below:

Lob Ark: is a rogued orchard mix made up of selections of loblolly pine (*Pinus taeda*) from local populations in Arkansas, Oklahoma, northern Louisiana and northeastern Texas although the majority of selections were made in Arkansas.

Lob NC: is a rogued orchard mix made up of selections from coastal NC.

Slash "Susceptible and Resistant": are standard check lots used at the USDA Forest Service Resistance Screening Center in Asheville, North Carolina that have been proven to be more "susceptible" and "resistant", respectively, to fusiform rust than wild stand collections of slash pine.

Seed were germinated and transplanted into Ray Leach Supercell containers. These containers were fertilized with one-half concentration of Miracle-Gro just prior to transplanting. Seedlings were then maintained at 70^0 F in the greenhouse until inoculation at six weeks of age. The test consisted of two runs, three trays per run, twenty trees per tray for a total of 120 trees per seedlot. Runs were inoculated one day apart. The inoculum was prepared by infecting three-week-old northern red oak seedlings with a bulk mix of aeciospores from both slash and loblolly sources from Louisiana, Mississippi, Alabama, Georgia and Florida. Basidiospores were harvested after three weeks incubation on the oaks. An inoculum solution was prepared at a density of 20,000 basidiospores per milliliter. Seedlings were preconditioned in a holding area for 24 hours, sprayed with inoculum, then

incubated in a chamber with controlled humidity (near 100%) and temperature (70^0 F) for 24 hours. Trees were then placed in the same holding area for another 24 hours then transported to a greenhouse.

Three weeks after inculation the test was fertilized with a full concentration of Miracle-Gro and placed on a six week fertilizing schedule. Seedlings were evaluated and percent galled scored at six months after inculation. Seed were sown in June 1996, inculations made in mid-August 1996 and evaluations were done in late January 1997.

Detailed procedures for fusiform rust screening can be found in the Resistance Screening Center Procedures (Knighten et al. 1988).

"Percent galled" was the only trait analyzed and reported on here. Analysis of variance was used to determine whether there were statistically significant differences among species/seed source lots, between runs and to detect run by seedlot interaction. The latter was tested to determine the repeatability of run results in terms of seed lot ranking. A correlation of seedlot ranking between runs was also conducted.

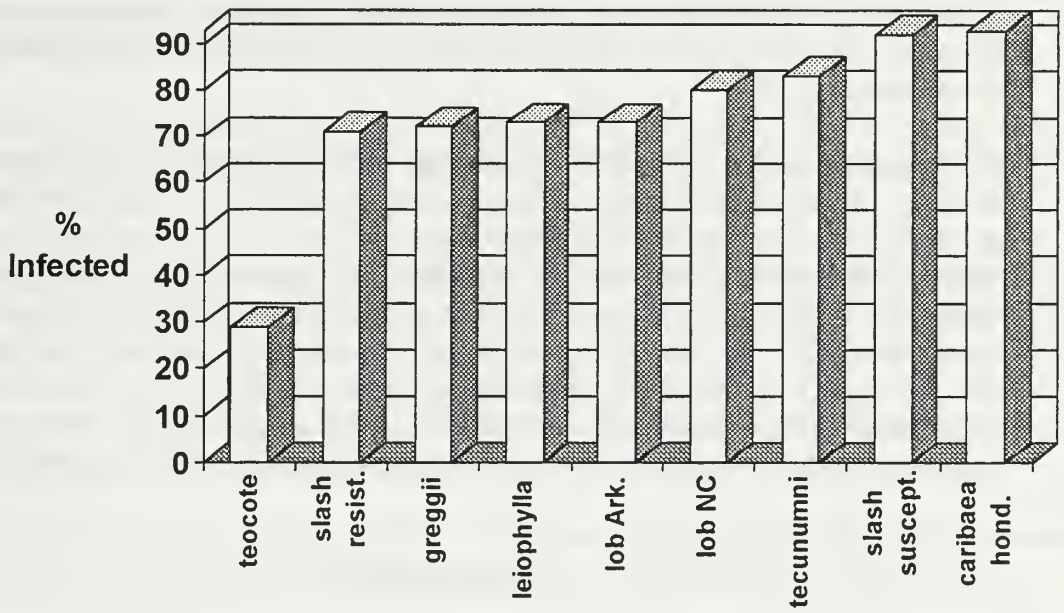
RESULTS AND DISCUSSION

Although the run x seed lot interaction was statistically significant at the 5% probability level, a high correlation of seed lot ranks between runs (r=.85) indicates that seedlots were consistent in their ranks for percent galled. When *Pinus leiophylla* Schl. et Cham. was dropped from the analysis the interaction term was no longer statistically significant and the correlation increased but only to r=.89. The overall fusiform rust infection level was high averaging 76% across all seedlots.

Pinus teocote had the lowest infection rate by far with only 29% infected (Figure 1) in spite of the high overall infection rate for all species. This species has morphological characteristics similar to shortleaf pine and may be evolutionarily related to it. Shortleaf pine is also highly resistant to fusiform rust and, when crossed with loblolly pine, conveys that resistance to the F1 hybrid progeny and to the backcrosses of the hybrid to loblolly (Kraus 1986). *Pinus teocote* would be a good candidate for hybridization with either loblolly or shortleaf pines. If it is otherwise adapted to the southeastern U.S. climate, it may confer good growth rate to either or both species and/or fusiform rust resistance to loblolly pine.

The next best non-improved species for fusiform rust resistance was *Pinus greggii* with 72% infection for the two provenances which were similar in their infection levels. The Valle Verde and San Joaquin provenances had 70% and 75% infection, respectively. These sources are from central Mexico and exhibit large differences in monoterpenes (Donahue et al. 1995), productivity (Dvorak et al. 1996b), and RAPD molecular markers (Furman 1997) to populations from northern Mexico. Because of these differences, trees from northern populations of *P. greggii* may be more resistant to fusiform rust than the southern provenances tested in this study. Both the northern and southern populations of *Pinus greggii*

Figure 1. Fusiform rust infection levels of seedlings of five Central American and Mexican pines, loblolly and slash pines at the Resistance Screening Center.



may also be good candidates for hybridization with loblolly pine since crosses may convey fusiform rust and drought to the progeny. *Pinus greggii* has shown excellent growth rates and good adaptability in subtropical regions (Dvorak et al. 1996b).

The slash pine "resistant" check lot had as low an infection rate (71%) of any species in this study except *Pinus teocote*, indicating the value of genetic selection for resistance in a species that is generally susceptible to fusiform rust. On the other hand, the "susceptible" slash pine lot had nearly as high an infection rate (92%) as the most susceptible species in the study which was *Pinus caribaea* var *hondurensis* with 93% infection. Tainter (1993) also found *Pinus caribaea* var *hondurensis* to be more susceptible to fusiform rust than slash pine. The infection rates were generally lower in Tainter's study but the species tested ranked similarly with *Pinus greggii*, "resistant" slash, "susceptible" slash and *Pinus caribaea* var *hondurensis* exhibiting infection rates of 53%, 58%, 75% and 88%, respectively.

Tainter (1993) tested some other Central American and Mexican pine species that may be of interest as exotics or as hybrids with our local pines. *Pinus patula* Schiede & Deppe in Schl. et Cham., *Pinus pseudostrobus* Lindl. and *Pinus oocarpa* Scheide had relatively low fusiform infection rates at 30%, 33% and 48%, respectively.

CAMCORE members are currently conducting a number of exploratory hybrid crosses between Mexican and southeastern U.S. pines to find those that are viable. Preliminary results suggest that these crosses may be more successful when conducted in subtropical environments rather than temperate ones. Obviously, it would be necessary to field test any new hybrid since phenology patterns and other factors could result in different ranking of species for fusiform rust resistance than those observed in this study where all seedlings were

at a highly succulent state at the time of inoculation. Nonetheless, these results can be useful in terms of selecting some potentially good candidate species from the large number of exotic pine species available for hybridization with local pines if fusiform rust resistance is of great importance. It would also be of great value to test these exotic species for resistance to the Nantucket pine tip moth (*Rhyacionia frustrana*) and other pests of commercial importance in the southeastern U.S..

Besides pest resistance, tolerance of tropical and subtropical pines to cold will be important in the choice of species to hybridize with local temperate species. Most of the exotic pines in this study (with the exception of *Pinus caribaea*) can be found at high elevation where freezing temperatures occur (Perry 1991) suggesting a certain degree of cold tolerance but those areas usually do not have the wild swings in temperature that can occur in the southeastern U.S.. One strategy for achieving cold tolerance between a local temperate pine, such as loblolly, and a relatively cold intolerant exotic pine may be to hybridize the exotic pine with a more northerly provenance than the one in the area of interest. For example, cross a Virginia provenance of loblolly with *Pinus teocote* for testing in South Carolina or Georgia.

CONCLUSIONS

1. *Pinus teocote* was by far the most resistant species in the trial with a fusiform rust infection rate of only 29% while the other species had infection rates of 70% to 93%.

2. The most susceptible species was *Pinus caribaea* var *hondurensis* with 93% infection.

3. Selection for fusiform rust resistance in slash pine has been very effective at reducing infection levels. The "resistant" check had 71% infection while the "susceptible" check had 92% infection.

4. These results illustrate the importance of fusiform rust resistance screening of exotics for hybridization with local species or for planting in areas where the alternate host (*Quercus* spp.) of the rust exists and where the disease may be a problem in the future.

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INHERITANCE OF BRANCHING AND CROWN TRAITS AND THEIR RELATIONSHIP TO GROWTH RATE IN LOBLOLLY PINE

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Abstract:-- Sweep, branching and crown traits were measured in ten-year-old loblolly pine (*Pinus taeda* L.) progeny tests for the purpose of studying inheritance patterns, for comparing them with growth characteristics and to study their genetic interrelationships. Branching and crown traits were analyzed as branches per whorl, branches between whorls, whorls per unit of height, branch diameter, live crown width, branch angle, branch diameter per unit volume, volume per unit of live crown width, and branches per unit of height. Of these traits, only branch angle, live crown width and volume per unit crown width were moderately heritable (greater than 0.10).

Genetic correlations with volume were generally favorable such that selection for volume should result in flatter branches, fewer branches per unit of height, greater stem volume per unit of crown width and lower branch diameter per unit of volume. Genetic correlations between North Carolina and Mississippi for branching and crown traits exhibited values less than 0.5 for all traits except branch angle, which had a correlation of 0.7.

Branch angle is the only trait which showed a consistent decline (perhaps a random effect) over two generations of selection, *i.e.*, branch angle for improved material was steeper than for unimproved. While live crown width and branch diameter increased for improved material, this increase was more than offset by gains in stem volume such that volume per unit of live crown width increased and branch diameter per unit of volume decreased.

Key Words: *Pinus taeda*, tree improvement, branching, straightness, crown.

INTRODUCTION

In general, U.S. loblolly pine (Pinus taeda L.) improvement programs have not chosen to improve branching and crown characteristics through targeted selection programs. Most often, during selection for volume in tree improvement cooperative programs, some phenotypic selection for crown form has been practiced but with minor weighting. Realized gain from this phenotypic selection for crown form has not previously been estimated.

Improvement in branching and crown characteristics is of interest to decrease knot size and frequency resulting in enhanced value for solid wood products. Knotiness produces increased grain distortion, pitch deposition and compression wood and reduces wood strength and

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usually decreases appearance value (von Wedel et al. 1967). To undertake an improvement program for branching and crown characteristics, one must ascertain the genetic architecture of the traits and the measurement cost. From these factors, calculations can be made of the cost associated with a given amount of genetic alteration of the characteristic. Projecting benefits from the genetic alteration through to solid wood products would allow consideration of investment in genetic change in branching and crown characteristics. This study was designed to provide the background data necessary to determine the genetic architecture and measurement time of several branching characteristics.

MATERIALS AND METHODS

The four progeny tests measured in this study were in Kemper County, Mississippi (2), and in Jones and Beaufort Counties in North Carolina. The Mississippi tests were planted in 1986 at 2.1m by 3.4m spacing and the North Carolina tests were planted in 1985 at 2.4m by 3.0m spacing. The family material in the tests is open-pollinated coastal North Carolina families from Comfort, N.C. and Lyons, GA seed orchards. The design at each test is randomized complete blocks with eight replications and five trees per family per replication. The five trees were planted at random in the replications, i.e., noncontiguous plots.

Traits measured in the winter of 1995-1996 included height, diameter at breast height, sweep, branch angle, width of live crown, number of whorls, number of nodal branches, number of internodal branches and diameter of branches. Height in meters and DBH was measured in centimeters. Sweep was measured as the maximum deviation (in two centimeter classes) from a 2.4m straight-edge anywhere in the first four meters of height. For charting of sweep means it was assumed that the mean of each category is the midpoint of that category, i.e., the mean of the 0-2 cm category is 1 cm etc.. Branch angle (tallied in six fifteen degree classes with zero being the closest to horizontal) was measured at the first major whorl above three meters at the point where the branch met the stem. For charting of branch angle means it was assumed that the midpoint of the category represents the mean of that category, i.e. the mean of the first category is 7.5 degrees and the mean of second is 22.5 degrees, etc.. Width of live crown in meters was measured down the row and perpendicular to the row. A whorl was defined as at least three major branches located at the same height on the tree. Nodal branches were counted as total number of branches present at whorls. Internodal branches were counted as the total number of branches between whorls. Branch diameter was measured (in centimeters) on the three largest branches beginning at three meters and ending at four meters in height. Whorls, nodal branches and internodal branches were measured beginning at three meters and ending at seven meters in height.

These measured variables were used to create thirteen analysis variables. The analysis variables were branch angle, branches between whorls, branches per unit of height, branches per whorl, whorls per unit of height, diameter at breast height, mean branch diameter per unit of volume, height, mean crown width, mean branch diameter, volume, sweep and volume per unit of live crown width. The analysis model was

$$y_{ijkl} = \mu + T_i + B_{ij} + gca_k + t_i*gca_k + b_{ij}*gca_k + e_{ijkl}$$

where: μ is an overall mean,

T_i is the fixed effect location,

B_{ij} is the fixed effect block within location,

gca_k is the random effect general combining ability ~ $N(0,\sigma^2_{gca})$,

t_i*gca_k is the random effect of the interaction between location and gca ~ $N(0,\sigma^2_{tg})$,

$b_{ij}*gca_k$ is the random effect of the interaction between block within location and gca ~ $N(0,\sigma^2_{bg})$ and

e_{ijkl} is the random error associated with the l^{th} observation of the k^{th} gca in the j^{th} block of the i^{th} location ~ $N(0,\sigma^2_e)$.

The analysis produced restricted maximum likelihood estimates of the variance components (Giesbrecht 1983) and best linear unbiased predictions of the gca's (White and Hodge 1989). Individual tree heritabilities were estimated as $3\sigma^2_{gca}/(\sigma^2_{gca}+\sigma^2_{tg}+\sigma^2_{bg}+\sigma^2_e)$, assuming that the genetic correlation among open-pollinated siblings is between that of half-sibs and full-sibs (Squillace 1974) and breeding values were estimated as 1.5 times gca. Pearson product moment correlations among the predicted breeding values were used as estimates of genetic correlations. For genetic correlations estimated by Pearson product moment correlation, there may be some shrinking of the correlation for sets of breeding values known with little precision as in low heritability traits with few observations.

RESULTS AND DISCUSSION

Genotype-Environment (State) Interaction:

Genetic correlations between regions were generally less than 0.50 except for branch angle which had a reasonably high genetic correlation between regions of 0.69 (Table 1). These low correlations suggest genotype-environment interactions of sufficient magnitude that could impact tree improvement strategies. Consequently, heritability and genetic correlations are discussed on a regional basis and no analyses were done across regions.

Heritability:

The tendency is for heritabilities to be slightly lower in Mississippi than in North Carolina. Heritabilities for branching and crown traits were disappointing as a whole (Table 2). Of the crown traits, only branch angle and mean crown width are as heritable as the growth traits. Branches per whorl, branches between whorls and branch diameter as a function of stem volume had very weak heritability (<0.05) at both locations. Volume per unit of crown width had heritability estimates slightly lower than those for growth traits. Frampton and Huber (1995) found branches/m of height, whorls/m and branch angle to exhibit higher full-sib family and clonal mean heritabilities than those for growth rate in four-year-old rooted cutting trials in North Carolina. Their results may differ from these because their heritabilities are influenced by specific combining ability and/or an effect due to rooted cuttings versus seedlings in our study. McCrady and Jokela (1996) concluded that, among five families chosen for extremes in growth potential, there were significant

Table 1. Breeding value correlations for Mississippi and North Carolina, above and below diagonal respectively, and between regions, on the diagonal[a].

	Branch Angle	Intern. Br./Whrl	Branch./ m Ht.	Branch./ Whorl	Whorls/ m Ht.	DBH	Br.Diam/ Volume	Height	Crown Width	Branch Diameter	Sweep	Volume/ Tree	Volume/ CrownW
Branch Angle	0.69	.	0.05	0.25	-.25	-.26	0.26	-.26	-.39	0.11	-.17	-.27	-.08
Intern.Bran./Whorl	0.03		.	.	.	.	.	.	.	.	.	.	.
Bran./m of Height	0.01	-.25	0.46	0.28	0.83	-.20	0.26	-.56	-.51	-.28	-.10	-.30	-.02
Branches/Whorl	-.12	-.13	0.06	0.44	0.03	-.02	-.02	0.12	-.26	-.08	-.11	0.02	0.22
Whorls/m of Ht.	-.12	-.55	0.87	-.19	0.36	0.16	-.21	-.15	-.19	-.31	-.11	0.09	0.28
DBH	-.21	-.00	-.42	-.00	0.05	0.43	-.80	0.46	0.71	0.39	0.52	0.97	0.82
Branch Diam./Vol.	0.21	0.06	0.06	0.09	-.21	-.78	0.42	-.73	-.55	0.05	-.28	-.84	-.78
Height	-.26	0.07	-.54	-.09	0.09	0.79	-.84	0.46	0.49	-.03	0.02	0.63	0.50
Crown Width	-.33	0.07	-.47	0.02	-.12	0.74	-.40	0.62	0.49	0.51	0.49	0.73	0.24
Branch Diam.	0.22	0.26	-.27	0.12	-.33	0.39	0.00	0.10	0.47	0.22	0.31	0.36	0.09
Sweep	0.12	-.04	-.08	0.10	-.09	0.25	0.02	0.22	0.48	0.12	0.46	0.45	0.33
Volume/Tree	-.24	0.00	-.46	-.02	0.06	0.98	-.77	0.86	0.75	0.31	0.29	0.45	0.83
Vol./m of Crown	-.07	-.04	-.30	-.06	0.18	0.83	-.83	0.77	0.28	0.08	0.00	0.84	0.28

Branch Angle is branch angle in 15° units from 0, near horizontal, to 5, near vertical.
Intern.Bran./Whorl is number of internodal branches divided by number of whorls.
Bran./m Height is number of nodal plus internodal branches divided by four meters.
Bran./Whorl is the average number of branches per whorl.
Whorls/m of Ht is the number of whorls divided by four meters.
DBH is diameter at breast height in centimeters.
Branch Diam./Vol. is branch diameter in centimeters divided by stem volume in cubic feet.
Height is height in meters.
Crown Width is average live crown width, down the row and across the row, in meters.
Branch Diam. is the average diameter of the three largest branches in centimeters.
Sweep is sweep as deviation from an 2.4 m straight-edge in two centimeters classes.
Volume/Tree is stem volume in cubic m.
Vol./m of Crown is stem volume in cubic feet divided by average live crown width.

[a]Values on and above the diagonal are significant at $\alpha = 0.10$ if the absolute value is greater than 0.30.
Values below the diagonal are significant at $\alpha = 0.10$ if the absolute value is greater than 0.28.

Table 2. Individual tree heritability for growth, sweep and branching characteristics in four 10 to 11-year-old progeny tests in North Carolina and Mississippi.

	North Carolina	Mississippi
Volume	.20	.13
DBH	.21	.14
Height	.17	.08
Sweep	.25	.12
Branch Angle	.18	.14
Crown Width	.17	.15
Volume per m Crown Width	.14	.10
Branch Diameter	.08	.02
Branch Diameter per Cubic m Volume	.04	.04
Number of Whorls per m Height	.08	.06
Branches per Whorl	.03	.04
Internodal Branches per Whorl	.02	.00
Branches per m Height	.09	.04

differences among families for height growth but none for most branching characteristics after four years in the field. Other conifer species have shown the tendency for high heritability of branch angle but low to moderate heritability for branch diameter and frequency (Adams and Morgenstern 1991; King et al. 1992)

Genetic Correlations Among Traits:

There are several significant trait-trait, breeding value correlations within regions (Table 1). Branches per m of height has several significant correlations with growth components, being negatively correlated with height, mean crown width and volume in both Miss/Ala and North Carolina. Branches per meter is strongly correlated with whorls per m and not correlated with branches per whorl, suggesting that branchiness is primarily a function of variation in whorls per meter. The significant negative genetic correlation of branches per unit of height and mean crown width may suggest that foliage may be displayed either by increasing the number of branches or by increasing the branch length.

Branch angle is moderately, negatively correlated with crown width and growth for both regions such that flatter branch angles tend to be associated with wider crowns and bigger trees. Whorls per unit height is significantly and negatively associated with mean branch diameter in both regions while being uncorrelated with growth rate. Branches per unit height in both regions is positively associated with whorls per unit of height while being negatively associated with growth traits and mean crown width.

Mean crown width and mean branch diameter are associated with growth rate in both regions such that larger trees tend to have wider crowns and larger branches. Sweep has positive and moderate correlations with volume and mean crown width in both regions. This association implies that genetically superior volume growers tend to have slightly greater sweep.

The genetic correlations of crown and branching characteristics with volume indicate that selection for volume will result in fewer branches per unit of height, larger mean crown width and larger mean branch diameter. The relationships of volume with mean crown width and mean branch diameter are mitigated when considering the results for volume per unit of crown width and mean branch diameter per unit of volume. The results for volume per unit of crown width and mean branch diameter per unit of volume suggest that, while larger crown width and mean branch diameter are associated with larger stem volume, selection for volume will, on average, produce more stem volume per unit of crown width and smaller branch diameter per unit of volume. Trousdell et al. (1963) found similar results for loblolly.

Internode length is genetically independent of volume so that selection for internode length could proceed while maintaining volume gains. Increased internode length would provide more clear wood between nodes for shop grade lumber. This gain in internode length would be difficult without increasing branch diameter and gains may be slow due to little variation.

Branch angle and internode length are the two branching and crown characteristics that are not genetically correlated with volume. Since these characteristics are uncorrelated with volume, selection for volume allows these traits to fluctuate at random; thus the need to consider selection for these traits. Other branching and crown characteristics are genetically associated with volume so that favorable responses should result from volume selection.

Genetic Variation:

Besides heritability and intercorrelation among traits, it is also important to know how much exploitable variation there is in a breeding population in order to know how the trait will respond to selection and to determine how much gain can be made in seed orchards. Plots of some different generation and orchard production types are plotted in Figure 1 in order to illustrate the degree of variation for six traits of interest. In the interest of brevity not all traits are plotted and only results from the North Carolina trial are shown but the general results in the Mississippi trials was the same. The best and worst full-sibs are averages of the breeding values of the two best parents and the two worst parents, respectively. Differences between these two bars on the graphs indicate the degree of variability still left among potential crosses in the 2nd generation group of parents. They also illustrate the potential for improvement if one is able to make controlled crosses on a commercial scale.

The trends were similar to those for heritability of the same traits, i.e., traits with low heritability have little exploitable variation. There was surprisingly little variation in branches per whorl, whorls per meter of height, branch diameter and (though it is not graphed) branches per meter of height. There is still considerable variation and potential gain to be made in volume, sweep and branch angle. Sweep has the greatest relative variation left in the population in spite of significant gains after two generations of selection. Branching characters as a function of tree size still have considerable variation. The only traits where the worst possible full-sib cross was not worse than the unimproved check was volume,where the worst full-sib was equal to the unimproved check, and volume per meter of crown width where the worst possible cross was better than the unimproved check.

Figure 1. Genetic variation for various genotypes in coastal North Carolina, 10 to 11-year-old tests. **Unimp** = unimproved check, **1st-Gen Rog** = mix of families from a rogued 1st generation seed orchard, **2nd-Gen Unrog** = mix of families from an unrogued 2nd generation seed orchard, **Worst and Best Full-sib** = Average breeding values of the worst two parents and best two parents in the 2nd generation, respectively, or what could be achieved with controlled mass pollination in an orchard.

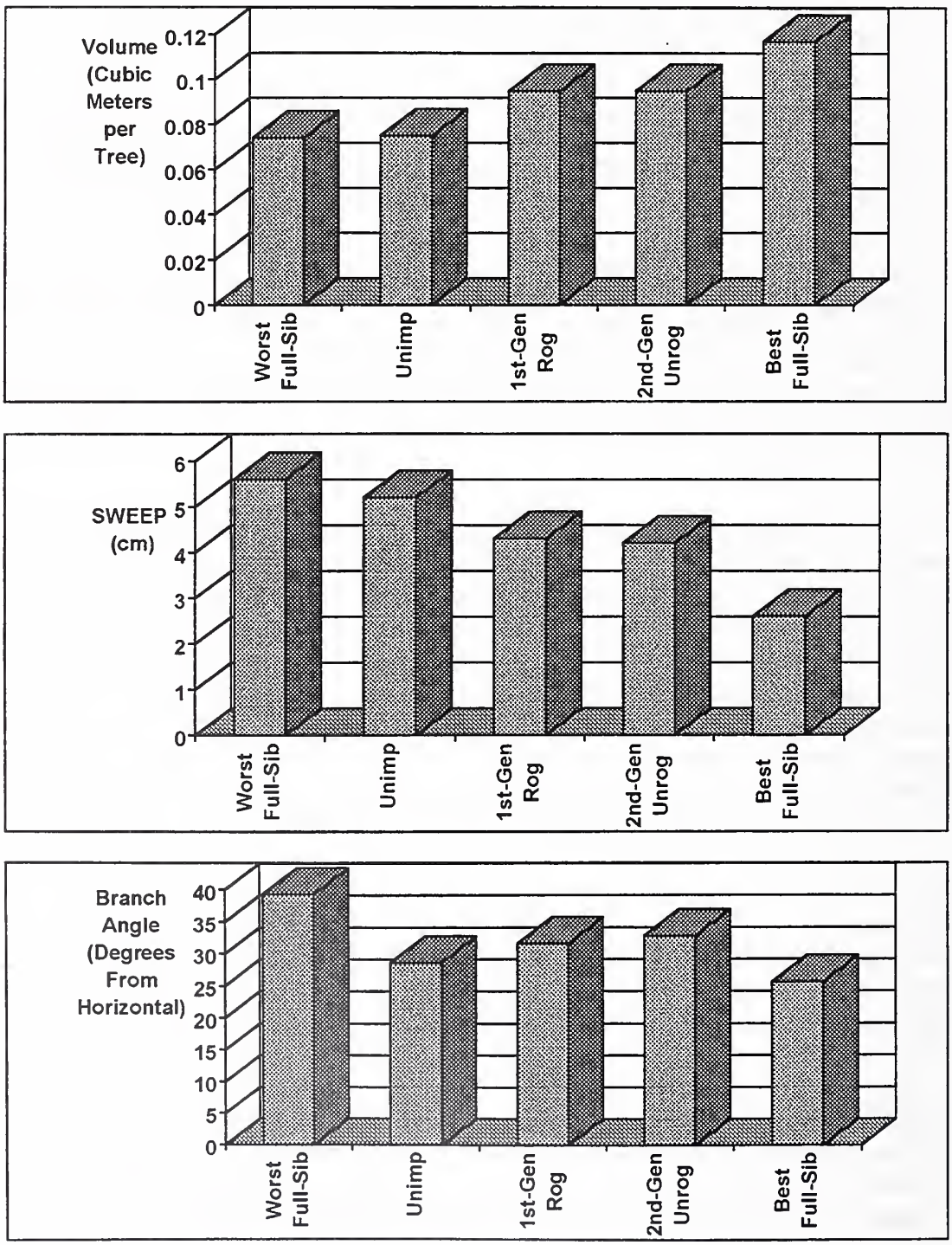
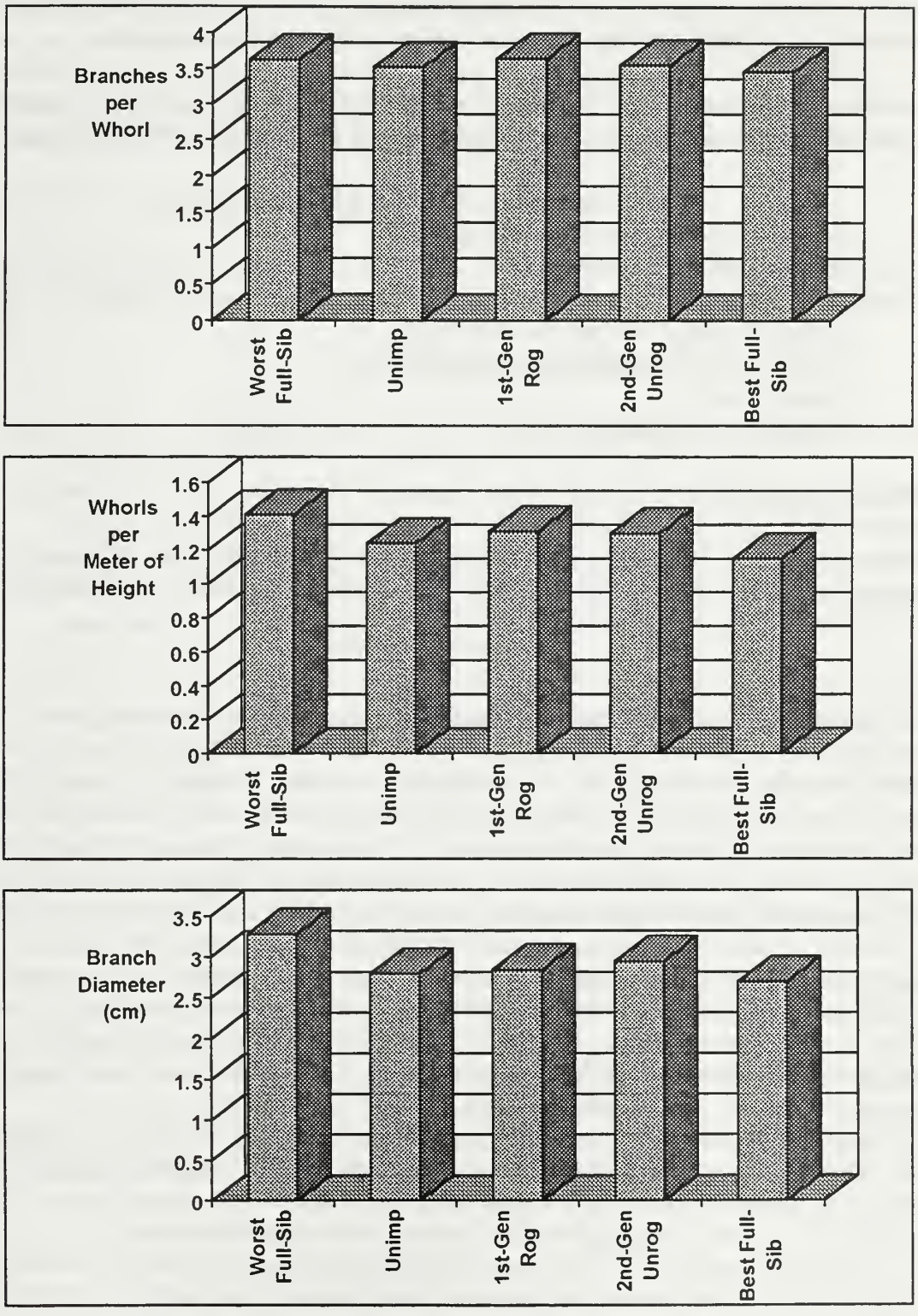


Figure 1. (cont.)



Seed Source Differences:

In the Mississippi trials first generation orchard checks of both local and coastal North Carolina provenances were included (Table 3). In general, the branching and sweep traits of the N.C. coastal source were more favorable than those of the central Mississippi source. Campbell, et al. (1995) reached the same conclusion about these two sources

Table 3. Differences between first generation, orchard mix check lots for N.C. coast and central Mississippi seed sources at age 10 in two tests in Kemper county Mississippi.

	Mississippi Source	N.C. Source
Branch Angle (degrees from horizontal)	34	27
Branches per Meter of Height	5.5	5.8
Whorls/Meter	1.2	1.8
Branch Diameter cm/Cubic m of Volume	.92	.75
Volume Cubic Meters/Meter of Crown Width	.94	1.02
Sweep (cm)	5.1	4.4
Volume Cubic Meters per Tree	.092	.098

Measurement Time:

Measurement time for the total of branching and crown traits was twice that of typical progeny test measurement which includes height, DBH, rust, condition code and sweep.

CONCLUSIONS

1. Heritabilities for several important branching traits such as branchiness, whorl frequency and branch diameter were significantly lower than those for stem height, diameter and volume. These branching characteristics also exhibited little genetic variability to exploit for genetic gain. Branch angle and crown width had heritabilities similar to those for height and volume and there is considerable genetic variation in branch angle that could be exploited if so desired. Sweep had moderate heritability and variability for further improvement in spite of the fact that improvements in through two generations of selection have been impressive.
2. Branch number, branch diameter and branch angle as measured in this study took twice as long to measure as the usual height, diameter, fusiform rust, condition code and sweep.
3. Branch angle was weakly but negatively correlated with growth rate (bigger trees tending to have flatter branch angle). In spite of the correlation, the second generation check had steeper branch angle than the first generation check which had steeper branch angle than the unimproved check, perhaps due to a random effect.
4. Bigger families tended to have wider crowns, and larger branch diameter in absolute terms but, when adjustments were made for size, they tended to have smaller branches and narrower crowns for their size and fewer branches per meter of height than smaller families. Therefore, selection for growth rate should result in improved branching characteristics.
5. In the Mississippi trials (there was no Mississippi material in the N.C. trials) the North Carolina coastal seed source had generally better quality characteristics with flatter branch

angle, less sweep, smaller branches for their size, slightly more volume per crown width but slightly more branches per meter of height than the local seed source.

The results of this study indicate that, barring significant economic impact for branching and crown traits, selection for volume is sufficient for these genetically correlated traits. The economic impact of the traits not genetically correlated with volume (internode length and branch angle) should be evaluated. Sweep should continue to receive weight in selection processes because of a modest but positive correlation with volume. The weights should be chosen according to the relative economic importance of growth rate and straightness.

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POPULUS TREE IMPROVEMENT IN NORTHERN INDIA

S. B. Land, Jr.[1] and N. B. Singh[2]

Abstract:--The status of tree improvement for *Populus* species in the hills and alluvial plains of northern India (above 28° N latitude) was assessed during two visits by the senior author in 1996. *Populus deltoides* Bartr. ex Marsh. var. deltoides from the USA is economically the most important Poplar species, but additional superior-performing clones need to be developed. Selection and studies of genetic variation in the indigenous poplars have started slowly and need to be accelerated, but hybridization work with *Populus ciliata* Wall. ex Royle appears promising for the hills. An All-India Coordinated Project on Poplar Improvement (AICPPI) has now been organized by the Indian Council of Forestry, Research & Education to coordinate and direct poplar improvement in the country, and Dr. N. B. Singh has been appointed director of the project. Priorities will be breeding *Populus deltoides* for agroforestry in the alluvial plains of northern India, developing indigenous poplars and hybrids for the hilly region of northern India, and introducing and testing subtropical and tropical poplars for agroforestry in central India.

Keywords: *Populus deltoides* Bartr. ex Marsh. var. deltoides, *Populus ciliata* Wall. ex Royle, India.

INTRODUCTION

The objective of this paper is to report the current status of *Populus* tree improvement research, development, and use in northern India. Information was obtained by the senior author from interviews, field trips, and reference materials during two visits to India in 1996. The visits were part of a consultancy activity between Winrock International Institute for Agricultural Development and the Indian Council of Forestry, Research and Education (ICFRE). Dr. N. B. Singh was the Indian counterpart from ICFRE for the consultancy.

USE OF POPLARS IN INDIA

Poplars are primarily used in agroforestry systems on the Indo-gangetic plains north of 28 degrees N latitude (Figure 1) and, to a lesser extent, in forestry plantations on the hills of north

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India. There is potential for their use in the valleys of the northwestern Himalayas, in the plains and hills of northeast India, and (possibly) for portions of east-central India north of 22 degrees N latitude (states of Madhya Pradesh [=M.P.], Bihar, Orissa, and West Bengal). The crop mixtures used in the agroforestry systems are described later in this paper.

Populus deltoides Bartr. ex Marsh. var. deltoides (typical eastern cottonwood from the USA) is an exotic and is currently the species of choice for agroforestry in the Indo-gangetic plains of the states of Uttar Pradesh (U.P.), Haryana, and Punjab. It is also the candidate species for use in east-central India, and may eventually be an important component of poplar culture in northeast India. Indigenous Indian species of *Populus* do not tolerate the heat of the plains (the possible exception is *Populus euphratica* Oliv., but this species grows slowly and has a bushy form). Little is known about *Populus gamblei* Dode in northeast India, but Dr. H. B. Naithani at the Forest Research Institute (FRI) in Dehradun, U.P., does not think that it can tolerate the high temperatures either. Hybrids of *P. deltoides* with the indigenous *Populus ciliata* Wall. ex Royle

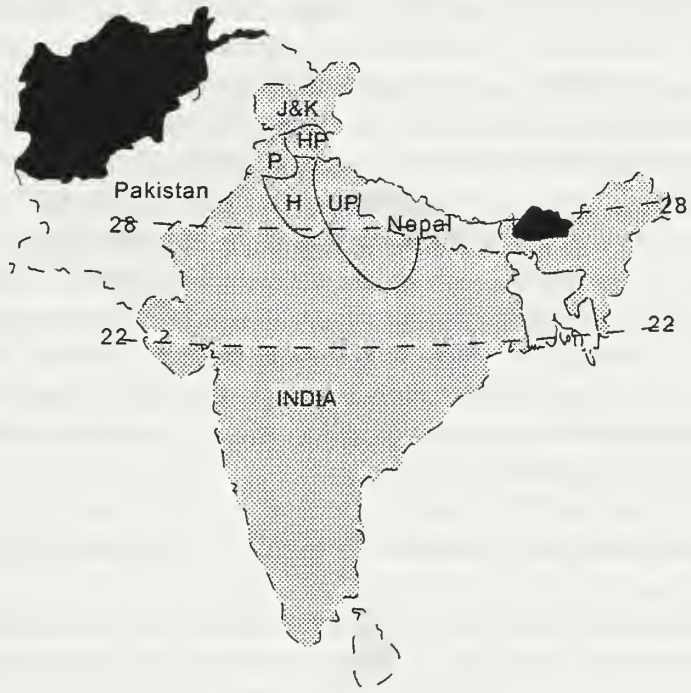

Figure 1. Locations of several states in northern India where Poplars are currently being planted. State abbreviations are J&K for Jammu and Kasmir, HP for Himachal Pradesh, P for Punjab, H for Haryana, and UP for Uttar Pradesh. Latitude 28 degrees North has traditionally been the southern limit for use of Poplars in India, but use of subtropical Poplar species may allow extension of this limit to 22 degrees North latitude.

exhibit heterotic growth in the nursery of the U.P. Forestry Department at Lalkuan, U.P., but fail (dieback) in agroforestry plantings on the U.P. plains by age three (personal communication with scientists at WIMCO Seedlings Ltd. near Lalkuan). A very robust market has developed for poplars grown in agroforestry systems on the Indo-gangetic plains. The primary products are

plywood, veneer, and matches, but secondary products include charcoal, fuelwood, leaf litter (composted), and (occasionally) fodder. Farmers are making money from these trees, and many now consider wood as their primary farm crop (personal communications with K. K. Sharma of ICFRE and Dr. J. P. Chandra at WIMCO).

The indigenous poplars, hybrids of *P. deltoides* with indigenous poplars, and other exotic poplar species and their hybrids with indigenous poplars are proving most suitable for reforestation/ afforestation in the hills of northern India. Dr. D. K. Khurana at the Y. S. Parmar University of Horticulture and Forestry in Solan, Himachal Pradesh (H.P.), recommends *P. deltoides* clones in the valleys below 900m elevation, the hybrid of *P. ciliata* x *P. maximowiczii* Henry on hills between 900m and 1500m elevation, and *P. ciliata* on hill sites between 1500m and 2800m (personal communication). He also reports that tests are being conducted further north in the state of Jammu and Kashmir (J&K) at Shere Kashmir University of Agricultural Sciences and Technology on the exotic species *Populus nigra* L. and *P. deltoides*, the indigenous species *Populus alba* L. and *Populus sauveolens* (?), and the hybrid of *P. deltoides* x *P. ciliata* (from his work). D. V. Negi with ICFRE at the Himachal Forest Research Institute (HFRI) in Shimla, H.P., reports that *P. alba* and *P. euphratica* only occur in limited, cold areas (high elevations) and might have potential for forest plantations above the *P. ciliata* plantings or for irrigated plantings in the cold desert region of H.P. and J&K. The U.P. Forestry Department is producing and testing some hybrids of *P. deltoides* x *P. ciliata* and *P. deltoides* with *Populus yunnanensis* Dode for use in the hills. Planting with poplars in the hills will be done almost entirely by State Forestry Departments on government lands without benefits of irrigation. Production will not be as rapid as for poplars in the agroforestry systems. Primary objectives shift from wood production to establishing a rapid tree cover for water and soil conservation. However, there is a local market for packing cases to serve the horticultural industry in H.P., so that poplar plantations on state lands might better serve this market than the imported eucalyptus cases from the plains. As the plantations mature, the plywood industry could expand into the hills to utilize the silvicultural cuttings from these plantations.

TREE IMPROVEMENT -- INTRODUCTION OF EXOTICS

At least 440 clones of various poplar species and hybrids were introduced and tested in northern India from 1958 to 1983 (Tiwari 1993). There were also some *P. deltoides* clones sent to India in the mid 1980's by Dr. Sam Foster (then in Louisiana and now with the U.S. Forest Service in Asheville, North Carolina), who made controlled crosses among some selected clones from Stoneville, Mississippi. These clones were tested by WIMCO Seedlings Ltd., but they did not perform well at early ages and were discarded. An additional large collection of approximately 200 *P. deltoides* clones from throughout the eastern United States was sent by Dr. E. A. Hansen of the U. S. Forest Service at St. Paul, MN, (now retired) to Dr. A. N. Chaturvedi of the U.P. Department of Forestry in January 1986.

Results indicate that *P. deltoides* from Texas and southern Mississippi River sources are best for the plains of northern India. These origins represent the southern 25% of the native range of *P. deltoides*, but clones have not been tested from origins east of the Mississippi River in the southern part of the range. *P. maximowiczii* and *P. yunnanensis* are other promising exotic

species (in the form of hybrids with *P. deltoides* or *P. ciliata*) for use in the mid-elevation range of the hills.

In 1990, Dr. D. K. Khurana at the Y. S. Parmar University of Horticulture and Forestry in Solan collected open-pollinated seeds from 103 *P. deltoides* trees along the Colorado and Brazos Rivers in Texas and along the southern part of the Mississippi River from Baton Rouge, Louisiana, to Davenport, Tennessee (Farmer and Khurana 1990, and personal communication with Dr. Khurana). He raised 50,000 seedlings at Solan and screened them down to 50 clones after three years of nursery measurements. Screenings were based on growth, stem form, and resistance to *Melampsora* spp. of leaf rust and to stem-borer insects. These 50 clones have been distributed to five state agricultural universities (in Punjab, Haryana, J&K, Orissa, and M.P.), two institutes (Tropical Forest Research Institute [TFRI] at Jabalpur, M.P., and Tata Energy Research Institute in New Delhi), and one private company (WIMCO Seedlings Ltd. in Rudrapur, U.P.). The oldest field trial (at Solan) was three years old in 1996.

TREE IMPROVEMENT--PROVENANCE INFORMATION ON INDIGENOUS POPLARS

There are six indigenous poplar species identified by Tiwari (1993) in India: *P. ciliata*, *P. alba*, *P. euphratica*, *P. gamblei*, *P. jaquemontiana* var. *glauca (?)*, and *P. laurifolia* Ledeb. Dr. D. K. Khurana at Solan also identifies a seventh species: *Populus sauveolens* (personal communication). These species occur in the hills of northern India, usually as scattered individual trees or as a few widely separated stands. The only provenance studies conducted to date have been for *P. ciliata*, *P. alba*, and *P. euphratica*, and almost all of the reported work has focused on *P. ciliata* in H.P.

Drs. P.K. Khosla and D.K. Khurana (both at the Y.S. Parmar University of Horticulture and Forestry in Solan) have initiated numerous studies on variation in *Populus ciliata*. They identified four ecological blocks ["types"] of natural occurrences of *P. ciliata*: (1) High-level association with fir-spruce, (2) Low-level association with blue pine (*Pinus wallichiana* A.B. Jacks) and deodar cedar (*Cedrus deodara* Roxb. Loud.), (3) Ravine/seasonal-water-source association with *Alnus*, *Ulmus*, and *Salix*, and (4) River flood basin/water channel sites on sandy soils (Khurana and Khosla 1982 and 1991). Phenotypic variation is greater among trees within stands than between stands within a type, but large differences are found between types. Provenance tests of *P. ciliata* that have been reported in the literature (Chauhan and Khurana 1992; Chaukiyal *et al.* 1995) involve cuttings from one clone (ortet) per source and from only nursery observations at one nursery near Solan. There is some evidence from these tests that better growth occurs from U.P. sources, but fewer limbs and better resistance to stem borers are found from J&K sources.

Recently, Dr. Khurana at Solan has established a larger provenance test of *P. ciliata* to rectify the problems of too few trees in the earlier tests (personal communication). He collected cuttings from 85 provenances (trees) throughout J&K, H.P., and U.P., and these are now 5 to 7 years old in field trials at three sites in H.P. He has also collected open-pollinated seeds from these same 85 trees, but the seedling clones have not yet been planted in field provenance tests.

D.V. Negi at HFRI in Shimla has recently completed a survey of *P. ciliata* in H.P. and *P. alba* and *P. euphratica* in H.P. and J&K (personal communication). In 1996, he collected and planted cuttings in Shillaru Nursery, H.P., from 124 trees (one male and one female tree in each of 62 stands) for *P. ciliata*, 32 trees (16 stands) for *P. alba*, and six trees (three stands) for *P. euphratica*. None of the *P. euphratica* cuttings survived. He plans to return to the trees (ortets) of *P. alba* and *P. euphratica* to collect open-pollinated seeds. This is part of a rangewide survey proposed to FAO[3] and coordinated by P. Khanna (FRI). Collaborators from FAO are Dr. J. B. Ball, Senior Forest Officer Plantations, and Dr. Oudara Souvawnavong (from Laos), who are located with the Forest Resource Development Service of FAO in Rome. Dr. H. B. Naithani (Botany Division, FRI) has been assigned responsibility for surveying *P. ciliata* in U.P. as part of this project. He has surveyed approximately one-half of the area in U.P. and has taken measurements on 200 trees. He will complete the survey in 1997, but has no plans to collect cuttings or seeds until so instructed by Mr. Khanna. The responsibility for a survey of *P. ciliata* and *P. gamblei* in northeast India has been assigned to Dr. Jasbir Singh and Mr. Sharma at the Institute of Rain and Moist Deciduous Forest Research (IRMDFR) in Jorhat, Assam. No accomplishments have been reported from that institute.

TREE IMPROVEMENT -- BREEDING (INCLUDING SPECIES HYBRIDIZATION)

Breeding involves crossing among selected trees to produce progenies with new combinations of genes for testing and selection. The resulting selections will be the clones of the next generation of improvement. Breeding requires flowering trees of the selected clones that will be used as parents. This is a major problem for exotics like *P. deltoides*, since almost no provisions have been made to establish and maintain breeding orchards of mature individuals of the introduced clones.

All controlled crosses involving *P. deltoides* before 1997 have been opportunistic, depending on what was flowering. Seven *P. deltoides* clones flowered during this time (G-48, D-121, and S7C8 were females, and G-3, S7C1, S7C15, and S7C20 were males). Except for D-121, all of these were from Brazos County, Texas. One male flowering clone of *P. yunnanensis* and one male of *P. 'robusta'* (Euramerican hybrid) were also used. "Breeding" in U.P. has been by the U.P. Forestry Department's Lalkuan Research Centre, Silviculture Division, Sal Region, by nearby WIMCO Seedlings Ltd. in Rudrapur, and at FRI in the Genetics and Tree Propagation Division. Initially, most of the new clones came from open pollinations between G-48 and G-3 (the first two clones to flower). These seedling progeny clones provided the "L-series" clones of the U.P. Forestry Department and most of the "WSL-series" clones of WIMCO. Subsequently, the U.P. Forestry Department nursery manager at Lalkuan made the following crosses (female x male): G-48 x G-3, D-121 x S7C1, G-48 x *P. ciliata*, D-121 x *P. ciliata*, G-48 x *P. yunnanensis*, and G-48 x *P. 'robusta'*. WIMCO selected three seedling clones from their own controlled crosses of G-48 x G-3, and they made some backcrosses (such as G-48 x *P. 'robusta'*) to stabilize desired hybrid combinations of *P. deltoides* with *P. nigra*. Controlled crosses by the

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[3] Noh, E.R., K.K.Sharma, and M.L.Kapoor. 1995. A report submitted to FAO on Improvement Programme of Indigenous Poplars with Particular Reference to India. FRI, Genetics & Tree Propagation Division, Dehradun.

Genetics and Tree Propagation Division at FRI were made in the spring of 1996 and required transport of flowering branches from Lalkuan. The crosses G-48 x G-3 and G-48 x *P. ciliata* were successfully accomplished. The *P. ciliata* male flowers came from the hills of nearby Mussoorie.

The greatest amount of controlled crosses among Populus species in India has taken place at the Y. S. Parmar University of Horticulture and Forestry in Solan, H.P. Flowers (male and female) of *P. ciliata* could only be successfully ripened on cuttings in the greenhouse if the cuttings were cleft-grafted to potted rootstock seedlings in January before bud break (Khurana 1989, Khurana and Thakur 1995). Water cultures and 'twig in pot' methods did not work. Cuttings of *P. gamblei* bearing male flowers could not be forced to ripen and shed pollen at Solan under any method, so no crosses with this species were made. Dr. Khurana successfully produced the hybrids *P. ciliata* x *P. maximowiczii* (pollen obtained from Japan), *P. ciliata* x *P. yunnanensis*, and *P. deltoides* (female) x *P. ciliata* (male) (but not the reciprocal) (personal communication). The *P. ciliata* x *P. maximowiczii* hybrid was heterotic for growth, but had the branch knot problem of *P. maximowiczii*. Dr. Khurana selected 20 clones from field tests of this hybrid for minimum branch knots.The *P. ciliata* x *P. yunnanensis* and *P. deltoides* x *P. ciliata* hybrids did not show heterosis for growth in his tests. Backcrosses of the Euramerican hybrid clone 'I-455' (female), which exhibits fast growth but is highly susceptible to *Melampsora* spp. of leaf rust, with *P. deltoides* (male) by Dr. Khurana produced two selections that exhibited extremely fast growth (4 to 5 meters per year in height) on recently-exposed, well-drained forest sites (not agroforestry) in H.P.

A total of 358 clones (primarily *P. deltoides*, including some intraspecific crosses of G-48 by G-3) have been preserved in a germplasm bank at FRI in Dehradun, U.P. There are also 277 clones of *P. deltoides* in a germplasm bank at the HFRI Shilly Research Nursery in Solan, H.P. Most of these are duplications of the clones at FRI, so that protection against a catastrophic loss of clones (from fire, etc.) is provided. The U.P. Forest Department's Lalkuan Research Centre is also maintaining a collection of clones from previous introductions and from inter- and intraspecific crosses. Many of these are duplications of clones at FRI. There are 150 clones in the Department's nursery at Lalkuan (100 *P. deltoides* clones), another 60 clones in a replicated test planted in February 1985 at 'Ganga Pur Patia East' near Lalkuan, and 233 clones in the Department's populetum planted in February 1989 at Tanda, U.P. (Plot #47). Forty-four of the clones in the Tanda planting came from Dr. Hansen's 1986 shipment of 200 clones to India and were selected based on nursery performance at the Lalkuan nursery. The Ganga Pur and Tanda tests were just beginning to flower in 1996 and will provide the only breeding orchard available in India (108 clones) for the initiation of an advanced-generation mating design with *P. deltoides*.

NURSERY AND GREENHOUSE TECHNIQUES

All *Populus* propagules being commercially planted in agroforestry or in reforestation/ afforestation are E.T.P.s (Entire Transplants). These are one-year-old rooted cuttings. The tree improvement program must therefore use E.T.P.s for clonal field trials.

Nursery management techniques include soaking the cuttings and nursery beds in water before planting and then planting the 18-25cm length cuttings at a spacing of 80cm x 60cm. Debudding to remove young limbs on the E.T.P.s is done from June to November. The nursery beds are flood irrigated 1-2 times per week during March - June, until the rainy season begins. Backpack sprayers are used to apply insecticides for control of leaf beetle, thrips, and wood-borer insects. The target E.T.P. for production nurseries is a plant whose ground-line diameter is 1/100th of the height (in cm) (personal communication with Dr. J. P. Chandra). When harvesting the one-year-old E.T.P.s in December - February, the tap root is cut at a 25cm depth and all side roots more than 10cm long are trimmed (Chaturvedi 1982, Sidha *et al.* 1990). The plants are packed, transported, sold, and planted with a naked root.

Research nurseries have the additional task of developing uniformity among E.T.P.s of the same clone. Re-propagating E.T.P.s annually from the previous year's E.T.P.s for 4-5 years in the nursery may be required to remove "C-effects" (age, position-in-tree, or vigor effects on cuttings taken from the crowns of different-aged ortets on different sites). The clones will not be taken to the field trials until within-clone variation is less than 10-15% (personal communication with Dr. Khurana at Solan). Furthermore, the multiplication of seedling-derived clones will take at least three years in the nursery to produce enough E.T.P.s for field trials at 2-3 sites.

When seeds are produced from controlled crosses during breeding, they must be germinated and grown before they can be vegetatively multiplied as E.T.P.s. *P. ciliata* seeds are very fragile and will lose viability in 7-10 days if not germinated quickly (personal communication with Mr. D. V. Negi). All *Populus* species seedlings are vulnerable to sun scald (heat) damage and diseases (damping off) during germination. Procedures for germinating and growing seedlings at WIMCO Seedlings Ltd. provide a working model for success. Fungicide-treated seeds are planted in rows on moist, heat-sterilized sand in flat clay pots and placed in a double-walled polygreenhouse with fans. The polygreenhouse is covered with 50% shade cloth. Approximately 15 days after the seeds have germinated, the germinants are 'pricked' and transplanted to individual cells in container racks. The soil in the containers is a barnyard mixture of manure that has been sterilized, so no additional fertilization is required. Containerized transplants are placed in a mist chamber in the greenhouse for one week (mist for 1-2 seconds at 5-minute intervals), then moved into a shadehouse for one week and subsequently outside under the shade of trees for 15 days. Finally, the acclimated seedlings are placed in the open sunlight where they remain until they are 30cm tall. At this size the roots can retain soil in a 'plug' when removed from the container. The 'plug' seedlings are then planted in the research nursery (personal communication with Dr. S. Chauhan of WIMCO).

AGROFORESTRY

The entire agroforestry industry with *Populus* is founded on a very limited genetic base. Approximately 90% of poplars being planted for agroforestry in U.P., Haryana, and Punjab come from the *P. deltoides* clones G-48, G-3, and S7C15 (personal communication with Dr. J. P. Chandra of WIMCO Seedlings Ltd.). The next two clones in popularity are 'Udai' from WIMCO and L-34 from the U.P. Forestry Department. Both of these clones come from open pollinations between G-48 and G-3. Other clones that are not quite as desirable, but which are being held in

reserve in case of a serious disease or insect outbreak, are S7C8, S7C4, L-43, ST-240, ST-70, and ST-67. All of these clones (except the three ST clones) came from gene pools in Brazos County, Texas. The three ST clones came from the southern Mississippi River alluvial plain just north of Vicksburg, Mississippi USA.

Typical agroforestry methods are to plant 4m-5m tall E.T.P.s in (1) "bund" (shelterbelt) plantings on the small levees around flood-irrigated agricultural fields, (2) "block" plantings at 5m x 5m, 5m x 4m, or 6m x 4m spacings within agricultural fields (underplanted with the crops), or (3) "row" plantings that contain alternating rows of Poplars and horticultural tree species underplanted with agricultural crops. Typical spacing between "rows" is 6m, so the *P. deltoides* rows are 12m apart. Rotation lengths for poplars are 5-8 years (5 years for plywood, 8 years preferred for veneer and matches). Typical crop combinations with "bund" plantings are sugarcane or rice, but rice is not recommended because of the requirement for summer flooding. Crop combinations with "block" plantings involve sugarcane for the first two years and then winter wheat or a combination of winter wheat alternating with summer-grown tumeric or pearl millet during the third through eighth years of the *P. deltoides* rotation. A new alternative to tumeric or pearl millet is celery. Dr. N. B. Singh has also observed rice being cultivated in some block plantings during the fourth through eighth years. Apparently, *P. deltoides* can tolerate flooding at the older ages. "Row" planting mixtures in agro-horti-forestry systems are based on a rotation length of up to 50 years for the final horticultural orchard. These systems may involve poplar-peach, poplar-litchi, or poplar-mango combinations with sugarcane, tumeric, and winter wheat as under crops. Up to two 5- to 8-year rotations of *Populus* may be grown before the fruit trees (particularly mango) spread in crown diameter to become a full orchard. In all of these systems the poplar trees are pruned of lower limbs (recommended lower 1/3 of stem height after the second year). The leaves are collected and composted at the end of the growing season for placement back on the fields, and the stumps are dug up and removed for fuelwood/charcoal at the end of the rotation. The above information was obtained from field visits and personal communications with Dr. R. P. Singh (HFRI), Mr. K. K. Sharma (ICFRE), and Dr. J. P. Chandra (WIMCO Seedlings Ltd.).

SUMMARY COMMENTS AND RECENT DEVELOPMENTS

There has been much plant material, expertise, and work involved in *Populus* tree improvement for India. However, there has also been little coordination of effort or continuity of guidance, since long-term assignments to projects have not been common. Although the widespread testing of many introduced clones, release of a few selected clones, and development of a new income source for farmers is worthy of praise, much more should have been accomplished in 40 years. Dr. Khurana in Solan has been very astute in seeing and working on the tasks that were most needed: (1) breeding of superior clones of *P. deltoides* and a few other exotic poplar species that were already present and proven in northern India (*i.e.* "land races"), (2) provenance testing of indigenous poplars for performance and for amounts and patterns of geographic genetic variation, (3) introduction of a larger gene pool (through seed collections) from the portion of the natural range of *P. deltoides* that has proven to be most productive in northern India, and (4) widespread testing of new clones from breeding, provenance testing, and new introductions across all of northern and east-central India.

The Indian government has recently recognized the need for central coordination of forestry research and development activities and has assigned this task to the Indian Council of Forestry, Research and Education (ICFRE). Desirable steps have already been taken. In mid 1996 the Director General of ICFRE appointed Dr. N. B. Singh to be Chief Technical Advisor (Coordinator) and Mr. Dinesh Kumar to be Associate Technical Advisor for an All-India Coordinated Project on Poplar Improvement (AICPPI). These two scientists are located at FRI in Dehradun, which is where ICFRE administration is headquartered. Priorities will be:

(1) advanced-generation breeding of 'land-race' clones of *P. deltoides* and introductions of new collections of *P. deltoides* from the southeastern USA for agroforestry in the alluvial plains of northern India (above 28 degrees N latitude), (2) developing indigenous poplars (*P. ciliata and P. gamblei*) and their hybrids with *P. yunnanensis* and *P. maximowiczii* for the hills of northern India, and (3) initiating introduction trials of subtropical and tropical poplars (*P. ilicifolia* Rouleau from the Tana River of tropical Kenya and *P. mexicana* subsp. *mexicana*, *P. mexicana* subsp. *dimorpha*, *P. fremontii* subsp. *mesetae*, and *P. guzmantlensis* from subtropical Mexico) (Pryor 1992) for agroforestry between 22 and 28 degrees N latitude in central India.

Several activities have already been accomplished at FRI during December 1996 through March 1997. A National Poplar Germplasm Bank with 350 clones has been established, 45 promising clones of *P. deltoides* have been planted in a breeding orchard, control crosses of *P. deltoides* have been made among six female clones and 15 males, and cuttings of 20 *P. deltoides* clones have been supplied to 15 cooperating research institutions for multilocational trials.

Perhaps with the new AICPPI the long-term commitment of resources needed for a *Populus* tree improvement program can be sustained. Such an approach is necessary to efficiently achieve the main goal of continually providing better planting material and products for the nation.

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GENETIC GAINS OF SECOND GENERATION SELECTIONS FROM THE NCSU-INDUSTRY COOPERATIVE TREE IMPROVEMENT PROGRAM

Bailian Li, Steve McKeand, Alice Hatcher and Robert Weir

Abstract: Data from 93 open-pollinated progeny tests of second-generation selections were analyzed to obtain genetic parameter estimates and breeding value predictions for height, volume and rust resistance. The 623 selections were grouped into four major geographic regions: 1) Virginia and northern North Carolina with 124 parents, 2) Atlantic Coastal Plains of North Carolina, South Carolina and Georgia with 131 parents, 3) Piedmont regions of North Carolina, South Carolina and Georgia with 285 parents, and 4) Lower Gulf region with 83 parents. The best linear unbiased prediction was used to estimate breeding values for 8-year height, volume and rust infection at a 50% infection level (R-50). The estimated gains for height from 2nd-generation seed orchards in the four regions ranged from 13% to 21% over unimproved checklots. Roguing these seed orchards intensively to the best 10-15 parents could boost the gains to as much as 20-24% in height growth and an even greater improvement in volume production at harvest. A substantial number of 2nd-generation selections have not only demonstrated outstanding growth but also had less rust infection, R-50 values of 20% to 25% below the unimproved checklots. The best family from each population was generally 10-20% above the 1st-generation seed orchard mix in height growth, indicating additional gain above the 1st generation selections. Although genetic gain for stem straightness is difficult to quantify, most of the 2nd-generation families had a higher percentage of trees with above average straightness than the checklots.

Keywords: breeding value, genetic gain, open-pollinated family, second-generation, seed orchard, rust infection.

INTRODUCTION

The N.C. State University-Industry Cooperative Tree Improvement Program has completed 41 years of loblolly pine genetic improvement. Members of the Cooperative, currently 16 industry members and five state agencies, annually plant 600 million trees on 900,000 acres accounting for 37% of the total annual tree planting in the U.S. The impact on forest productivity has been substantial through the two cycles of breeding, testing and selection by cooperative members. Trees grown from seeds of first-generation seed orchards have produced 8-12% more volume per acre at harvest than the trees grown from wild seed (Talbert et al. 1985). With additional improvement in value from quality traits (stem straightness, disease resistance, wood density),

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the estimated genetic gain in plantations from first-generation breeding is about 20% (Weir and Todd 1993). Second-generation seed orchards are now producing more than 50% of the total seed harvest in the region. Data from open-pollinated progeny tests of these 2nd-generation seed orchards are now available to provide genetic gain estimates.

MATERIALS AND METHODS

Second-generation selections were made from progenies of incomplete factorial matings of the 1st-generation parents where each of 20 to 30 females was mated to four to six males. These selections were grafted to establish 2nd-generation seed orchards by each cooperative member. Open-pollinated seeds from each seed orchard were collected, and 2nd-generation progeny tests were established by each member organization throughout the Southeast. The number of families in each test series ranged from 19 to 44 including several check seedlots. Families common to multiple seed orchards were included in several test series within each region. Each test series generally included 4 tests established over a two year period in two locations. The experimental design was a randomized complete block with six blocks and 6-tree row plots. All tests (93 in total) were measured for tree height and some were measured for DBH, stem straightness and rust infection. Since open-pollinated loblolly pine families generally show little genotype by environment interaction and high family stability in performance across a wide geographic area (Li and McKeand 1989, McKeand et al. 1990), the tests were grouped into four general geographic regions (Figure 1): 1) Virginia and northern North Carolina with 124 families in 5 test series, 2) Atlantic Coastal of North Carolina, South Carolina and Georgia with 131 families in 5 test series, 3) Piedmont regions of North Carolina, South Carolina and Georgia with 285 families in 12 test series, and 4) Lower Gulf region with 83 families in 3 test series.

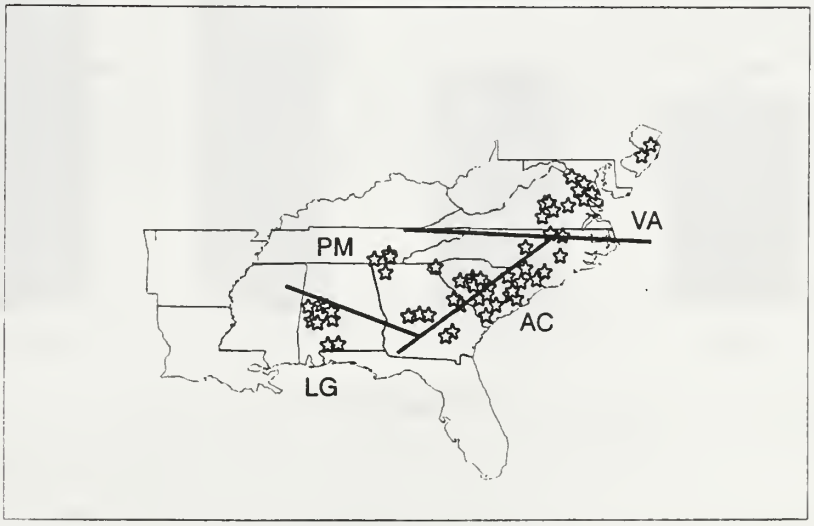

Figure 1. Distribution of 2nd-generation open-pollinated tests in four geographical regions. Note: VA=Virginia and northern NC, AC=Atlantic Coastal, LG=Lower Gulf, PM=Piedmont.

All data were adjusted for age and scale effects before estimating the genetic parameters and breeding values in each region. The best linear unbiased predictions (BLUP) was used to estimate parental breeding values because the data are unbalanced with different parents, ages and test qualities (Huber 1993). The BLUP for parental general combining abilities (GCA) were estimated and then breeding values were estimated using the GCA estimates. Breeding value estimates were based on 8-year height and volume measurements. For ranking parents within a given geographic region, percent genetic gain over local checklots was calculated from the predicted breeding values for height. Breeding values for rust infection at a 50% infection level (R-50) were also calculated for ranking parents for rust resistance.

RESULTS AND DISCUSSION

Family-mean heritabilities for individual test series ranged from 0.51 to 0.87 for height and from 0.43 to 0.87 for stem volume. The family-mean heritabilities for rust infection varied with the test infection levels and ranged from 0.01 to 0.86. Breeding value estimates are summarized by regions for percent gain over local checklots for height (Figure 2). Only height gain is presented here because not all tests had DBH and rust measurements.

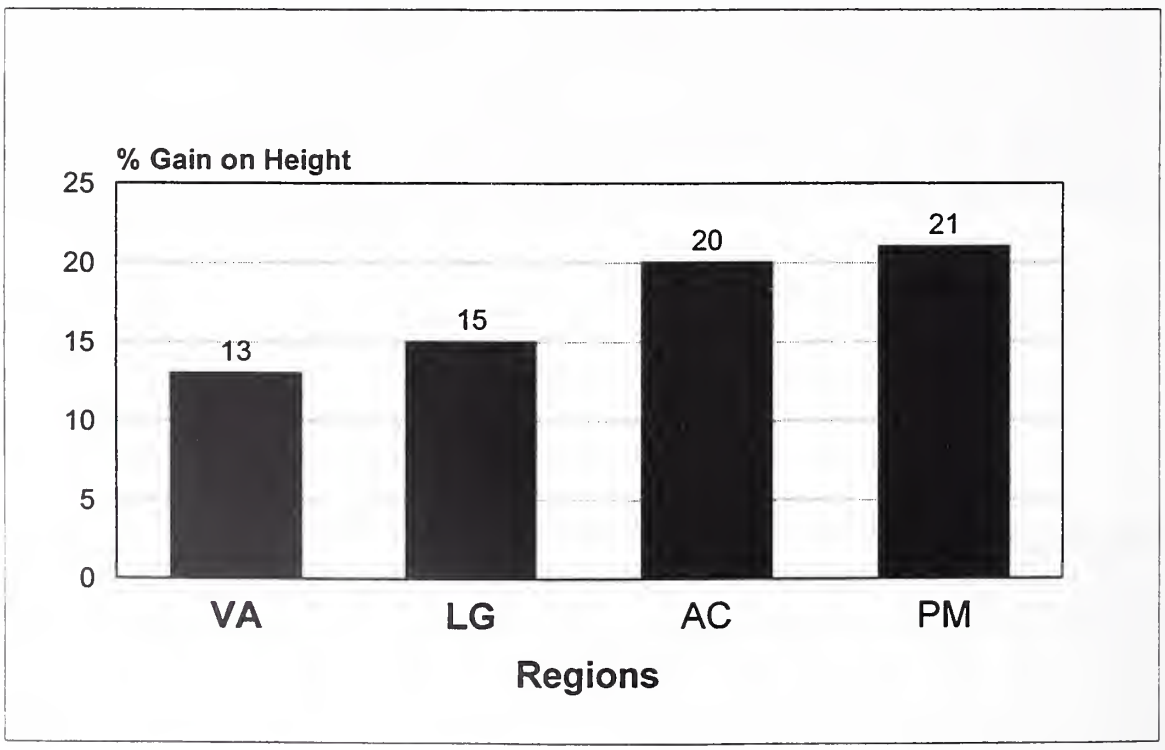

Figure 2. Genetic gain estimates (%) for 8-yr height over local unimproved checklots for the four geographical regions, VA=Virginia and northern NC, AC=Atlantic Coastal, LG=Lower Gulf, PM=Piedmont.

It is evident from these estimates that 2nd-generation selections have produced substantial gains over unimproved checklots. The genetic gain for 8-yr height from the top 30% of families was 13% above the local checklots for Virginia/NC, 15% for the Lower Gulf, 20% for the Atlantic Coastal Plain, and 21% for the Piedmont region. The gain for the Piedmont families ranged from 11.6% over the NC Piedmont checklots to 20.9% over the Piedmont checklots of SC. Volume gains over unimproved checklots should be greater than those based on height (Talbert et al. 1985). These estimates indicated that, in general, second generation breeding and selection has been effective for improving loblolly pine growth even with the limited selection intensity due to the tester mating design used in the 1st-generation breeding program.

Rust infection (R-50) was generally lower for 2nd-generation families than for the checklots. For example, in the Atlantic Coastal population, about 80% of the families had lower R-50 breeding values than all three checklots. The top ranked 30% of families for rust in the Atlantic Coastal population had an R-50 of 29.6%, significantly lower than the three checklots (above 63%). Similar differences in R-50 were observed for the Piedmont population which averaged 28% for the best 30% families and 56% for checklots. No strong correlations were found between height growth and R-50 breeding values except in the Lower Gulf population. While rust infection was generally high for tests in the Lower Gulf, the R-50 was moderately correlated ($r=-0.48$) with height growth. Because of this favorable correlation, it is possible to select fast growing families with relatively low R-50 values.

Much greater genetic gains can be expected from utilizing the best families since large differences were observed among 2nd-generation families. The best Atlantic Coastal family had 31.6% gain over the unimproved checklots of NC and 17.1 over the checklots of SC, while the best Piedmont family had 29.3% gain over the unimproved checklots of SC. The best family from each population was generally 10-20% above the checklot of 1st-generation seed orchard mix, indicating additional gains above 1st generation selections. These gain estimates were based on open-pollinated progeny. Additional genetic gains could be achieved by selecting a few outstanding parents and making controlled mass pollination, assuming that specific combining ability is negligible for a full-sib cross. Although genetic gain for stem straightness is difficult to quantify because of different ages and scoring systems in different tests, it is evident that most of the 2nd-generation families had a higher percentage of trees with above average straightness than the checklots.

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ULTRASTRUCTURAL CHANGES DURING EARLY DEVELOPMENTAL STAGES OF SOMATIC EMBRYOS IN LOBLOLLY PINE (PINUS TAEDA L.)

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Abstract:--Ultrastructural changes of somatic embryo at early developmental stages were studied in loblolly pine (Pinus taeda L.). Proliferation and maturation media of Gupta and Pullman (1991) have been used to obtain embryogenic tissues and somatic embryos, respectively. Some degenerating and declined suspensor cells were observed in well-maintained embryogenic tissues. This may explain why the embryogenic cultures have to be subcultured frequently and sometimes lose embryogenic ability. In stage 1 embryos, starch granules started to accumulate in plastids, but lipid bodies showed no significant increase. In comparison to the stage 1 embryos, embryos at stage 2 contained increased number of lipid bodies. Cytoplasm of embryonic cells was also richer in free ribosomes, which may be an early sign of storage protein synthesis. Vacuoles in stage 1 and 2 embryos were larger and/or more numerous than that of embryonic cells in maintained embryogenic tissue. Lack of osmoticum in maturation medium was suspected as one of the potential causes of vacuolated embryonic cells with no protein accumulation.

Keywords: lipid body, somatic embryo, storage protein, suspensor cells

INTRODUCTION

Somatic embryogenesis in conifers has been extensively studied (Becwar et al. 1995; Tautorus et al. 1991). However, the main effort in this area has primarily focused on mass and rapid production of somatic embryos due to its high commercial value. In conifers, detailed ultrastructure of developing zygotic embryos has not been studied (Tautorus et al. 1991) except in Douglas fir, Pseudotsuga menziesii (Owens et al. 1993). Also, the ultrastructure of somatic embryos has been studied only in white spruce, Picea glauca (Fowke et al. 1990; Hakman et al. 1987) and European larch, Larix decidua (Rohr et al. 1989). In contrast to the success in Picea and Larix species, difficulties still remain in efficiently producing mature somatic embryos in loblolly pine (Pinus taeda L.). The objective of the present work is to study the ultrastructural changes during early development of somatic embryos. This research will provide cellular information of somatic embryo development, which may help to improve embryo maturation in loblolly pine.

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MATERIALS AND METHODS

Plant material. Seed cones of loblolly pine were collected at Hope, Arkansas, on June 30, 1993. Seeds were sterilized by the method described by Li and Huang (1996). Intact megagametopyhtes containing immature zygotic embryos were excised from seeds with a sharp scalpel and used as explants for initiation of embryogenic tissue. The explants were laid on the surface of culture medium in petri dishes. One of initiated embryogenic cell lines, named as H_{10} , was a clonal product from an explant. This cell line had been maintained for about one year and subcultured at least 20 times before it was used in this ultrastructral study.

Initiation and proliferation of embryogenic tissue. The composition of basal medium used in this experiment was described by Gupta and Pullman (1991) except that the concentration of boric acid was doubled to 31 mg/liter (Li et al. 1996). The initiation medium (BM_1) was supplemented with 50 μM 2,4-Dichlorophenoxy acetic acid (2,4-D), 20 μM kinetin, 20 μM 6-Benzylaminopurine (BA) (Gupta and Pullman 1991). The initiated embryogenic cultures were then transferred to BM_3 medium for proliferation, the basal medium plus 5 μM 2,4-D, 2 μM kinetin, 2 μM BA, and 9,000 mg/liter myo-inositol (Gupta and Pullman 1991). Translucent and mucilaginous embryogenic tissues containing both embryonic and suspensor cells were excised from the surface of culture and used for the ultrastructural study. The embryogenic tissues were healthy and vigorously growing, and had potential to show all the developmental stages, from the cell masses to mature somatic embryos, which were dependent on the composition of culture medium and duration of culturing time.

Stage 1 and 2 somatic embryos. The staging system for somatic embryo development followed the work of von Arnold and Hakman (1988). Stage 1 and 2 embryos are defined as:

Stage 1 embryos are small embryos consisting of an embryonic region subtended by an elongated suspensor.

Stage 2 embryos are bullet-shaped embryos with a clear prominent embryonic region that is more opaque than stage 1 embryos.

To induce stage 1 and 2 embryos, embryogenic tissues were transferred to maturation medium (BM_4), the basal medium supplemented with ABA at 80 mg/liter and activated charcoal at 1 mg/liter (Gupta and Pullman 1991). After culturing about a month on the maturation medium, stage 1 somatic embryos were grown about 0.5 to 1 mm long. Somatic embryos at stage 2 with a length of about 2 mm were formed in about 2 months. Somatic embryos at both stages were studied under a transmission electron microscope. The samples for the electron microscopy were selected from cultures raised on solid media.

Electron microscopy. Small pieces of embryogenic tissue and somatic embryos at different developmental stages were fixed in a modified Karnovsky's fixative consisting of 2% glutaraldehyde and 2% paraformaldehyde in 0.2 *M* cacodylate buffer at pH 7.4 for 2 hr in a

weak vacuum and then washed with 0.05 *M* cacodylate buffer at pH 7.2 twice. This procedure was followed by post-fixation in 1% OsO_4 in the same buffer for 2 hr. The tissues were then prestained with 0.5% aqueous uranyl acetate at 4° C overnight, dehydrated stepwise in a graded ethanol series (30 to 100%) and propylene oxide two times to ensure complete dehydration. The specimens were then infiltrated with the mixture of 50% to 50% propylene oxide-Spurr's embedding medium (Spurr 1969) for 2 hr. The specimens were finally embedded in 100% Spurr's medium and polymerized in an oven at 70 ° C overnight. Ultra-thin sections cut with a LKB ultramicrotome were stained with 2% uranyl acetate for 4 min, followed by lead citrate at pH 12.0 for 2 min (Reynolds 1963).

RESULTS

Embryogenic tissue. Embryogenic tissue contained two types of cells, smaller embryonic cells and long suspensor cells. The smaller embryonic cells at this stage were tightly attached together. Nucleus generally occupied a large area in the center of embryonic cell and contained two nucleoli, but nucleoplasm was clear. Plasmodesmata occurred between embryonic cells. Some very small vacuoles were observed in the cytoplasm. Mitochondria were abundant and varied in shape. Golgi bodies were scattered in the cytoplasm and were primitive. Plastids usually contained starch granules and had only a few thylakoids, if any. Endoplasmic reticulum (ER) was mostly of a rough variety. A few lipid bodies were scattered in the cytoplasm while no typical protein bodies and only a few starch granules in plastids were observed in embryonic cells at this stage.

The long and large suspensor cells were characterized by having a large central vacuole, which reduced cytoplasm to a thin parietal layer. The suspensor cells were loosely attached each other, and no intercellular connections or plasmodesmata were observed. Cytoplasm also contained abundant mitochondria. Plastids had varied profiles. The ERs were mostly of a rough variety. The cytoplasm of suspensor cells was as dense as that of embryonic cells, and Golgi bodies were also very primitive. One striking phenomenon of embryogenic tissue was that a considerable number of suspensor cells showed various signs of degeneration. The cytoplasm first became electron-lucent due to the loss of matrix substance; Golgi cristae became dilated and contained numerous small membranous vesicles while some of the other organelles, such as mitochondria and plastids, appeared still intact. In some cells, the plastids and mitochondria collapsed and the nuclei were disintegrating.

Stage 1 somatic embryos. Embryonic cells at this stage were smaller but had denser cytoplasm than that in the maintained embryogenic tissue. The cells in embryonal apical dome showed the outer cell wall with a cuticle. Cells along the outside had thicker cell wall than those positioned in the center. Embryonic cells were more closely associated with each other at this stage. There were numerous plasmodesmata between the thin cell walls. Many of the nuclei contained two to four nucleoli and clumps of chromatin were much more abundant throughout the nucleoplasm in comparison to that of the embryonic cells at the previous stage. More numerous and larger vacuoles in the embryonic cells at this stage were formed in the cytoplasm compared to the embryonic cells in embryogenic tissue. Starch

synthesis appeared to be very active as indicated by the fact that a large number of starch granules were accumulated in plastids. The plastids showed early differentiation, indicated by the appearance of thylakoids. Ribosomes were also more abundant at this stage than at the previous stage. However, no storage protein accumulation was observed in the cytoplasm. The number of lipid bodies had no obvious change.

Stage 2 somatic embryos. The most significant phenomenon at this stage was an obvious accumulation of lipid bodies in embryonic cells. Starch granules were also abundant and accumulated in plastids. The accumulation of storage reserves suggested that the embryos were on the way toward maturation. Free ribosomes were also richer in embryonic cells at this stage than at the previous stages, indicating active protein synthesis, although the storage protein or protein body was not observed. Microtubules were also observed in the cells. However, small vacuoles had fused together and formed bigger ones.

DISCUSSION

Embryogenic tissue of loblolly pine was whitish to translucent in color and resembled embryogenic tissue of other conifers, but showed no embryo development on the proliferation medium. The embryogenic tissue was also called embryonal-suspensor cell mass and contained numerous very early stage embryos (Gupta and Durzan 1987). Each embryo had elongated cells at one end (suspensor) and smaller cells with large nuclei and dense cytoplasm at the other (embryonal) end. The little differentiated plastids in embryonic cells with dense stroma but very few thylakoids if any indicated that the tissues possessed embryogenic ability. Non-embryogenic calli generally contained chloroplasts, typically with numerous grana and large grains of starch; differentiated plastids in embryonic cells in embryogenic tissues indicated that the tissues had lost or were losing embryogenic ability (Rohr et al. 1989; Becwar et al. 1988).

The structurally unhealthy and degenerating suspensor cells found in embryogenic tissue were another striking phenomenon. It was not surprising, however, because a previous study showed that suspensor cells can be generally stained with Evan's Blue, while embryonic cell can be stained with acetocarmine (Gupta and Durzan 1987). This so-called double-staining technique has been used to identify embryonic and suspensor cells in embryogenic tissue in coniferous species (Gupta 1995). However, Evan's Blue is often used to determine cell or tissue viability (Gaff and Okang 1971). The cells stained by Evan's Blue are generally considered that they are dead or, at least, under degeneration. This study clearly showed that there were a considerable number of degenerating suspensor cells in the embryogenic tissue.

It has been demonstrated in Larix decidua that suspensor cells showed the advanced signs of senescence after the embryonal apical dome had been formed during somatic embryo development (Rohr et al. 1989). *In vivo*, suspensors started to degenerate as soon as zygotic embryos were extruded from archegonium by the support of elongated suspensor. However, embryos in embryogenic tissue were still at very initial development stage and suspensors

had not elongated yet. Suspensor cells should not start to degenerate at this stage, and various signs of senescence in suspensor cells may be abnormal. Degenerating and declined suspensor cells in embryogenic tissues may explain why the tissues needed to be subcultured frequently and sometimes lost embryogenic ability.

It was evident that starch among storage reserves first started accumulation in somatic embryo and this accumulation was followed by development of lipid bodies. No protein body was observed in somatic embryos at early stages. Presence of protein body in somatic embryos may not occur until the late cotyledonary stage (Smith, personal communication). This order in accumulation of storage reserves seems to be different from that of zygotic embryos. In Douglas fir, protein bodies were first observed in zygotic embryos although they were small and only a few (Owens et al. 1993). In comparison to stage 1 somatic embryos, no lipid bodies and starch were found in zygotic embryos at the similar stage. Later, accumulation of lipid bodies and starch started almost simultaneously. Protein bodies had not significant increased until very late developmental stage, but they were less abundant in zygotic embryos than in megagametophyte (Owens et al. 1993). Ultrastructural study of zygotic embryos in loblolly pine is lacking.

Highly vacuolated embryonic cells and a lack of protein bodies were consistently observed in stage 1 and 2 somatic embryos. They may be not an independent phenomenon but appeared to be associated with each other. Absence of osmoticum in maturation medium resulted in highly vacuolated embryonic cells, non-uniform embryo maturation and smaller cotyledon region in white spruce (Attree and Fowke 1993). High vacuole profile was also accompanied with lower storage protein and lipid contents in mature embryos of white spruce. Lack of osmoticum in the maturation medium, therefore, was suspected to be one of potential reasons for vacuolated embryonic cells with no protein accumulation; in turn, the early stage somatic embryos failed to develop to the cotyledonary stage in loblolly pine.

Seed storage proteins play an exceedingly important role in the reproduction and survival of angiosperm (Bewley and Black 1994). The storage protein has been observed during somatic embryo maturation in other conifer genera, such as *Picea* and *Larix* (Attree and Fowke 1993; Rohr et al. 1989). A richness of free ribosomes and a rough ER profile in stage 2 embryonic cells may indicate the onset of deposition of storage protein. Gene expression of storage protein has been demonstrated to be activated by ABA treatment (Skriver and Mundy 1990). However, both osmotic pressure and ABA controlled the gene expression of storage protein in angiosperm (Bewley and Black, 1994). In white spruce, it was evident that ABA initiated synthesis of storage protein, but osmoticum was needed to regulate storage protein synthesis at the post-transcriptional level (Attree and Fowke 1993). Osmoticum may also have an important role in regulating synthesis of storage protein in loblolly pine. In this experiment, the high osmolarity induced by myo-inositol was present only in the proliferation medium but was absent in the maturation medium. Cell line H_{10} has not produced cotyledonary embryos on this maturation medium with 2 to 80 mg/liter ABA in our lab. Increasing osmolarity of culture medium may reduce the extent of vacuolation of somatic embryonic cell and enhance protein accumulation; in turn, somatic embryo maturation will be improved. Later studies demonstrated that the addition of 3 to 10%

polyethylene glycol (MW 3,350) as an osmoticum induced cotyledonary somatic embryos for H_{10} and another cell line (Li et al. 1997).

CONCLUSIONS

Degenerating and declined suspensor cells found in well-maintained embryogenic tissues may explain why embryogenic cultures needed to be subcultured frequently and sometimes lost embryogenic ability. The order in accumulation of storage reserves in somatic embryos seems to be different from that of zygotic embryos. Although no protein body was observed, richness of free ribosomes and a rough ER profile in stage 2 embryo may indicate that storage protein synthesis had been activated. However, the stage 2 embryos have failed to mature further on the medium with only ABA. Lack of osmoticum may be one of reasons resulting in vacuolated embryonic cell without storage protein depositions. Since osmoticum has been evident having an important role in regulating storage protein synthesis and somatic embryo maturation, it deserves to be investigated for improving the maturation of somatic embryos in loblolly pine.

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SOMATIC EMBRYOGENESIS IN LOBLOLLY PINE (PINUS TAEDA L.)

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Abstract:--Factors affecting embryogenic culture initiation for loblolly pine have been determined, and a maturation medium that efficiently produces cotyledonary embryos has been developed. Basal medium, the combination of plant growth regulators, Phytagel™ concentration, potassium chloride, silver nitrate and myo-inositol showed significant effects on embryogenic culture initiation. For embryo maturation, polyethylene glycol and maltose synergistically promoted production of cotyledonary embryos. About a hundred cotyledonary embryos from a gram of embryogenic tissue can be potentially produced on this newly-developed maturation medium. The mature embryos had well-developed cotyledons and root cap.

Keywords: embryogenic culture, initiation, maltose, maturation, polyethylene glycol, somatic embryos.

INTRODUCTION

Forest productivity needs to be raised to satisfy the increasing demands for wood worldwide. The conventional method for reforestation of conifer species is primarily artificial regeneration with seedlings or by natural regeneration. Tree improvement is based on selection, establishment of seed orchard, and progeny testing. The conventional method for genetic improvement is a very slow process because of long generation cycles (Gupta and Durzan 1991), and is limited in genetic gain due to the variation among sibling seeds. Large-scale production of control-pollinated seeds is very expensive. Asexual propagation has been recognized as having great potential for capturing genetic advantage in tree improvement programs (Hall 1980). In loblolly pine, asexual propagation can be accomplished by grafting, rooting of cuttings and tissue culture. Grafting is used only to propagate superior individuals in a limited quantity. Vegetative propagation by cuttings is problematic in most coniferous species, including loblolly pine, due to rooting difficulty. Tissue culture methods, such as somatic embryogenesis, provide the potential for rapidly multiplying valuable genotypes for reforestation and will help in the race to increase forest productivity (Gupta et al. 1993). Tissue culture methods may also interface with genetic engineering techniques to regenerate transgenic plants.

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Somatic embryogenesis has been very successful in several coniferous species, especially in Picea species, whereas Pinus species are highly recalcitrant to this technique and success has been limited (Tautorus et al. 1991). Successful somatic embryogenesis in loblolly pine will be highly significant for reforestation and tree improvement programs.

Loblolly pine (Pinus taeda L.) is the most important timber tree in the southern United States and may be the second most dominant plantation timber species in the world (Gupta and Durzan 1991). During the past 4 years, we have used an Arkansas source of loblolly pine to initiate embryogenic cell lines, which were then used to develop an embryo maturation medium. A relatively efficient embryo maturation protocol has been developed. We present here a summary of research results.

INITIATION OF EMBRYOGENIC CULTURE

In loblolly pine, the initiation of embryogenic cultures has been solely from immature zygotic embryos, and the initiation frequency has been low, generally less than 10% (Becwar et al. 1990; Li and Huang 1996). Several factors affecting the initiation frequency were identified, including genotype (Becwar et al. 1990), maturity of zygotic embryos (Gupta and Pullman 1991), initiation medium (Becwar et al. 1990), plant growth regulators (Becwar et al. 1988), and concentration of gelling agents (Becwar et al. 1995). Several other factors, which potentially promoted initiation of embryogenic culture, have been identified in our laboratory, including potassium chloride, silver nitrate, myo-inositol or their combination at various concentrations (Li and Huang 1996).

DCR_1 (Becwar et al. 1995) and BM_1 (Gupta and Pullman 1991) are two standard culture media to initiate embryogenic cultures in Pinus species. To determine which is better for initiation for an Arkansas source of loblolly pine, the culture medium was divided into three parts: basal medium including organic and inorganic nutrients, plant growth regulators (PGRs) and gelling agent. We determined superiority of each part in both culture media.

The BM_1 basal medium was superior to DCR_1, with less browning and higher extrusion and proliferation frequencies. However, combination of PGRs used in DCR_1 standard medium showed better initiation results than those used in BM_1 standard medium. Phytagel level at 2 g/L, which is commonly used in tissue culture medium, achieved better initiation results than that at 1 g/L. Although 4 g/L Phytagel also showed promising results, a difficulty with further maintenance was observed later at this concentration. Therefore, 2 g/L Phytagel is recommended for initiation.

This study showed that both standard initiation media were not optimum for the selected loblolly pine genotypes. Since each medium has its advantages and drawbacks, they need to be further improved or should be employed with selection. Our study in exploring factors affecting initiation may also provide important information to develop more effective initiation media for other Pinus species.

MATURATION OF SOMATIC EMBRYOS

Maturation of somatic embryos is a very critical step in somatic embryogenesis of conifers. Except for Monterey pine (Aitken-Christie et al. 1994; Smith et al. 1994), Pinus species are highly recalcitrant to embryo maturation in comparison to Picea species (Tautorus et al. 1991). Although a total of ten U.S. patents related to somatic embryogenesis in loblolly pine have been claimed, embryo maturation is still very challenging and can not be consistently achieved. Abscisic acid (ABA) has been recognized as an essential component in maturation media to induce cotyledonary (bearing well-developed cotyledons) embryos in conifers (Gupta and Pullman, 1991; Tautorus et al. 1991; Uddin 1993). A high ABA concentration was generally used in Pinus species (Li et al. 1997; Becwar et al. 1995; Gupta and Pullman, 1991). However, in our genotypes, cotyledonary embryos were rarely induced with BM_4 medium (Gupta and Pullman, 1991), which contains up to 100 mg/L ABA (Li et al. 1997).

In our laboratory, we have identified two other factors that significantly affect embryo maturation in loblolly pine. One is osmotic potential and the other is the type of carbohydrate. The osmotic potential provided by polyethylene glycol (PEG) plays an important role in somatic embryo maturation (Li et al. 1997). Without PEG, embryo maturation frequently failed to occur on a medium containing sucrose. PEG combined with sucrose increased maturation frequency by at least tenfold. To induce cotyledonary embryos, a high concentration of maltose has been used to replace sucrose as a carbohydrate source and osmoticum (Uddin 1993; Becwar et al. 1995). However, we did not observe that maltose itself could induce a highly efficient production of cotyledonary embryos. Maltose and PEG did act synergistically to promote embryo maturation. Embryo maturation efficiency can be enhanced to about a hundred cotyledonary embryos based on one gram of embryogenic tissue at the proliferating stage when both polyethylene glycol and maltose were present in the maturation medium. When sucrose, maltose and PEG were present in the maturation medium, the maturation frequency was lower than PEG combined with maltose. Further, the optimum concentration of PEG and maltose in the culture medium was determined. We generally recommend that about 6% PEG and 4% maltose should be included in the maturation medium to achieve efficient embryo maturation (Li et al. unpublished data).

Maltose as a carbohydrate source also showed its superiority to sucrose in terms of morphology of cotyledonary embryos. The embryos produced from medium with maltose replacing sucrose generally were longer and had well-defined root caps. Therefore, maltose may be a more favorable carbohydrate than sucrose for embryo maturation, while polyethylene glycol is preferred over additional maltose as an osmoticum.

Currently, protocols efficiently inducing cotyledonary embryos in loblolly pine are still rare and not reliable for most genotypes. Here an alternative embryo maturation protocol is provided for loblolly pine. The basic strategy used in this study may also be employed for further improvement of embryo maturation medium, which hopefully can be used for more genotypes.

Although embryo germination has been achieved in our laboratory, germination frequency was not high and germinated embryos showed poor vigor for continuous growth. Plant establishment has not been achieved.

Previous research has indicated that partial drying or desiccation treatment enhanced germination frequency in white spruce (Attree and Fowke 1993). We found that most cotyledonary embryos failed to tolerate desiccation treatment, but over 90% did show viability following partial drying (water loss about 50%). Most surviving embryos showed elongation of cotyledons and hypocotyl, and some progressed to epicotyl growth. However, only limited embryos showed radicle emergence, and some did not elongate further after initial radicle emergence.

Several reasons may contribute to the failure of plant establishment. First, the quality of somatic embryos should be considered. Gupta and Pullman (1993) suggested that a lower concentration of ABA should be used at the later embryo development stage than at the early stage. However, other research results showed that plantlet establishment was achievable with steady ABA concentration during embryo maturation. We did not use stepwise-adjusted ABA concentration during maturation in this study, but some deleterious effects of medium with a high ABA level was evident on the further development of early cotyledonary embryos, such as swelling of the hypocotyl region and slowdown of cotyledon elongation. Generally, zygotic embryos at the cotyledonary stage contain a very low level of ABA (Kapik et al. 1995). Further studies need to determine whether maturation medium with a high ABA level affects the quality of somatic embryos and then the germination. Second, germination conditions may also impact the plantlet establishment, including culture medium, temperature, light, and humidity. Information regarding optimum germination conditions is lacking for Pinus species.

CONCLUSIONS

For initiation, both standard media for embryogenic culture initiation were not optimum for an Arkansas source of loblolly pine. To modify the formulations, basal medium and PGR combination and level should be considered. Lower concentration of Phytagel did not promote initiation. PEG as an osmoticum played an very important role in embryo maturation. Maltose was a better source of carbohydrate than sucrose in embryo maturation. And most importantly, PEG and maltose acted synergistically to enhance embryo maturation efficiency in loblolly pine. However, it is still difficult to germinate cotyledonary embryos and to establish vigorously growing plants.

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STEM CUTTING PRODUCTION AND ROOTING IN A SLASH PINE DIALLEL MANAGED FOR RAPID CUTTING PRODUCTION[1]

M.E. Mason[2] and C.D. Nelson[3]

Abstract: Seventeen crosses from a five-parent diallel mating design of slash pine were tested for stem cutting production and rooting ability. Seedling progeny were grown in pots, fertilized weekly, and hedged as soon as suitable length (> 7 cm) cuttings were available. A total of seven hedging and rooting cycles were completed in the first 14 months. Each seedling stock plant was scored in each cycle for the total number of usable cuttings produced and the percentage of cuttings set that rooted. Overall mean cutting production per seedling increased from 5.6 in cycle 2 (1.0 in cycle 1) to 15.8 in cycle 4, then fell to 10.4 in cycle 6. Overall mean percent rooting decreased from 67% in cycle 2 (95% in cycle 1) to 28% in cycle 5. The decrease in percent rooting was much more rapid than anticipated, and may reflect seasonal effects or a negative impact of the rapid-cycling management system. Cycles 1-4 (age=early) were characterized by increasing cutting production and good rooting (>55%), while cycles 5-6 (age=late) showed level to decreasing production and poor rooting (<35%). Family and age effects and family x age interaction were significant for both cutting production and percent rooting. Cumulative numbers of cuttings rooted per stock plant ranged from 4 to 95. Subsequent rooting trials have shown a reversal in the decreasing trend for cutting production and percent rooting, suggesting an opportunity for successful clonal multiplication using a rapid-cycling technique.

Keywords: *Pinus elliottii*, genetic variation, vegetative propagation, rooted cuttings

INTRODUCTION

Vegetative propagation has played an important role in the genetic improvement and analysis of southern pines. The routine ability to propagate selected trees by grafting has greatly accelerated the rate and level of improvement in operational tree breeding programs. Stem cutting propagation has been a useful research tool for analyzing the genetic control of important traits (Nelson et al. 1993a, Frampton and Huber 1995), but has largely failed to move beyond the research phase (McRae et al. 1993). Recent advances in macro- and micro propagation, as well as the increasing potential of gene transformation and genetic engineering, have increased the interest in vegetative propagation research and clonal forestry applications.

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Successful use of stem cutting propagation for research or production purposes depends upon the ability to efficiently multiply selected genotypes. Vegetative multiplication of pine and many other forest trees is limited by the reduction and near loss of rooting ability as clones age (Libby et al. 1974). Because of this limitation, early selection and clonal multiplication are critically important (Wülisch 1984). The greater the number of cuttings that can be rooted in the first year and developed into productive stock plants, the larger the final multiplication potential of the clone.

The Southern Institute of Forest Genetics (SIFG) has employed stem cutting propagation as a research tool for many years (Hare 1974, Nance and Nelson 1989). As part of ongoing research into the genetic interaction of fusiform rust disease (Jewell 1961, Jewell and Mallett 1967, Dinus and Griggs 1975, Griggs and Walkinshaw 1982, Nelson et al. 1993b), we have been vegetatively propagating seedling progeny from a five-parent diallel of slash pine. The initial propagation goal was to produce adequate numbers of rooted cuttings from each seedling to allow time and space replicated inoculations with a series of fungal isolates (Nance et al. 1992). A rapid-cycle hedge management system (Nelson et al. 1993a) was employed in an attempt to maximize cutting production in the first year from seed. This paper discusses the results of the first year's propagation efforts.

MATERIALS AND METHODS

Population

Parent trees were originally selected as superior wild trees for inclusion in early (1950s) breeding programs at the SIFG. Open-pollinated progeny were included in field tests and fusiform rust resistance screening experiments. Based upon results from these studies (Jewell 1961, Jewell and Mallett 1967), the trees were selected for inclusion in a diallel mating design for further disease resistance work (Griggs and Walkinshaw 1982). Control pollinations for the current work were conducted in 1989 and 1990. The mating design and number of seedlings tested per family are shown in Table 1. Seeds were collected, processed, and stored frozen until use in November 1991.

Stock Plant Initiation and Maintenance

Seeds were germinated on wet vermiculite in a growth chamber (25°C, 16 hour photoperiod) and transplanted to a peat:vermiculite mix in Ray Leach stubby cells (7 in^3). Seedlings were grown in a greenhouse (16 hour photoperiod) for 16 weeks before hedging was initiated. At 28 weeks, the seedlings were transplanted to one gallon pots containing a pine bark:peat:vermiculite mix and grown outside under ambient temperature and light conditions for the remainder of the experiment. Seedling stock plants received weekly fertilizer treatments of Peters 20-20-20 applied at 200 ppm nitrogen. Stock plants also received periodic insecticide treatment to control aphids and mites.

Table 1. Mating design, pedigree codes, and number of progeny tested per family.

♀/♂	18-27[1] (SIFG 1001)	8-7 (SIFG 1002)	9-2 (SIFG 1003)	18-26 (SIFG 1004)	18-61 (SIFG 1006)
18-27	11[2]/30[3]	12/10	13/30	14/20	15/35
8-7	21/35	22/30	23/15	24/20	25/35
9-2				34/20	35/15
18-26	41/20	42/20	43/20	44/10	45/30

[1]Tree identification, SIFG xxxx is the database clone number (Mason et al. 1993).
[2]family code, parents are coded 1-5 and families are coded as female x male (ie. 12 is 1 x 2).
[3]number of seedlings used in current experiments.

Cutting Collection and Rooting Conditions

Stock plants were hedged and cuttings were collected at approximately 6 to 8 week intervals beginning at 16 weeks from germination. On each cutting collection date, all potential stem cuttings were removed. A random subset (from 80 to 100%) of the usable (>7 cm) cuttings was set for propagation. Cuttings were treated with commercially available rooting powder containing 0.3% IBA, and set into a peat:perlite mix in Ray Leach fir cells (3 in^3). The propagation greenhouse was maintained at moderate temperatures (<90°F) and high humidity (>85%) using a combination of evaporative cooling, air conditioning, intermittent mist, and fog. After 8 to 10 weeks, the stem cuttings were scored for rooting and transferred out of the propagation environment.

Data Collection and Analysis

During each hedging cycle the number of usable cuttings (NrCut) per stock plant was counted. All shoots longer than about 7 cm were considered usable. In cycles 1, 3, and 5-7, all usable cuttings were set, while 5 and 10 cuttings per stock plant were set in cycles 2 and 4, respectively. After 8 to 10 weeks in the rooting environment, the cuttings were removed from the containers and inspected for rooting. For each stock plant, the number of cuttings developing one or more primary roots was recorded. Proportion rooted (pRoot) was calculated as the number of cuttings rooted divided by the number of cuttings set. The number of propagules (NrProp) was calculated as the product of the number of usable cuttings and the proportion rooted (NrCut*pRoot). Since either all or a random subset of the cuttings were set in each cycle, we assumed that the discarded cuttings would root at the same rate as those actually set. The number of usable cuttings produced were not recorded for cycle 5, so cycle 5 production values (NrCut) were estimated to be the same as cycle 4. Rooting data for cycle 7 were inadvertently lost, so proportion rooted was estimated to be the average of the proportion rooted for cycles 5 and 6. Statistical analysis were conducted using SAS version 6.11 (SAS Institute Inc. 1996). Diallel analysis were completed using the program DIALL (Schaffer and Usanis 1969).

RESULTS AND DISCUSSION

Overall rooting percentages varied from 95% in cycle 1 to 28% in cycle 5 (Table 2). Production values ranged from 5.6 in cycle 2 to 15.8 in cycle 4. A definite trend in proportion

rooted and cutting production was observed (Figure 1). Two distinct periods can be identified based on rooting and production. The early period (cycles 1-4) was characterized by increasing cutting production and rooting above 55%, while the late (cycles 5-7) exhibited level to declining production and rooting below 35%. This distinct change in propagation performance was noted previously (Nelson et al. 1993a) in a study examining rapid-cycle propagation in loblolly pine. We are uncertain as to the cause of this decline. Possible explanations include biological and/or cultural effects. In both experiments, seasonal effects (photoperiod and temperature) were confounded with seedling development. The rapid-cycle technique may have induced the decline due to imbalanced nutritional status and/or a negative effect from recurrent wounding and tissue loss. In any case the effect appears transient as these stock plants have performed well in later years of rooted cutting propagation (H.E. Stelzer personal communication).

Table 2. Overall Summary of rooting experiments by cycle. Bracketed values are estimates as described in the text.

Cycle	Estimated Age (weeks)	Average NrCut	pRoot	pSet	Number of clones set	Average cumulative NrProp
1	16	1.0	0.95	1.0	395	0.95
2	24	5.57	0.67	0.86	394	4.81
3	30	7.39	0.56	1.0	383	9.10
4	36	15.8	0.60	0.62	382	18.6
5	42	[15.2]	0.28	0.57	372	23.1
6	48	15.3	0.35	1.0	335	28.3
7	54	10.4	[0.33]	1.0	250	31.8

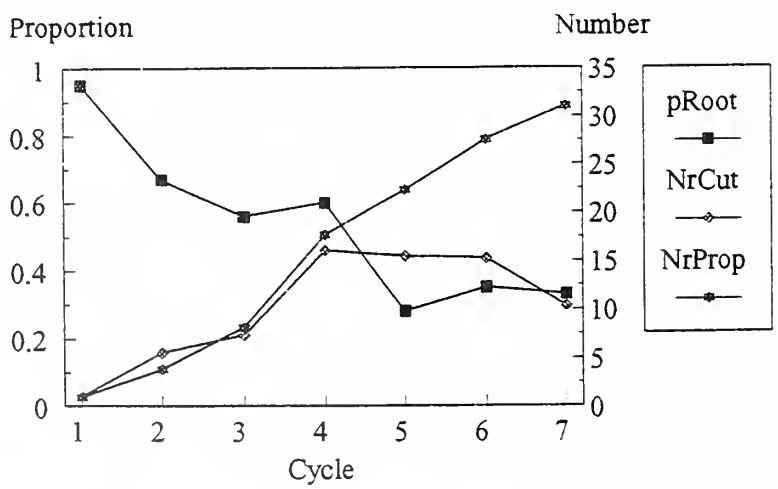

Figure 1. Plot of proportion rooted, number of cuttings produced, and cumulative number of propagules per stock plant for 17 families (395 clones) of slash pine.

The age by family interaction was significant ($p \leq .05$) for each trait (Table 3). For number of cuttings and number of propagules the interaction was primarily due to scale effects, as the error variance was much greater late than early (Table 4). Changes in rank were much

more important for proportion rooted, however the higher and lower ranked families early tended to remain so late, or move to the middle. No families moved from one extreme to the other. This was not the case, however, for clones-within-families (data not shown). Clones tended to change ranks to a much higher degree. In fact on a per cycle basis, many clones tended to alternate between higher and lower rooting percentages.

Table 3. Analysis of age and family effects on proportion rooted and number of cuttings and number of propagules.

		pRoot		NrCut		NrProp	
Source	df	ms	p>F	ms	p>F	ms	p>F
Age	1	15.1	***	23364	***	2621	***
Family	16	.130	***	2372	***	811.6	***
Age X Family	16	.051	**	768.2	***	121.0	*
Error	735:756:735	.024		208.1		64.3	

significance levels: *** ≤.001, **≤.01, *≤.05

Table 4. Analysis of family effects by age on proportion rooted and number of cuttings and number of propagules.

		pRoot		NrCut		NrProp	
Source	df	ms	p>F	ms	p>F	ms	p>F
Age=Early (cycles 1-4)							
Family	16	.053	***	307.6	***	239.4	***
reciprocal	1	.111	*	282.3	*	396.5	**
maternal-1, 18-27	1	.052		273.0	*	343.1	**
maternal-2, 8-7	1	.007		18.4		66.4	
maternal-4, 18-26	1	.023		171.1		123.0	
self vs outcross	1	.329	***	584.3	***	857.5	***
self-1, 18-27	1	.061		32.8		147.3	
self-2, 8-7	1	.404	***	915.9	***	1228	***
self-4, 18-26	1	.127	*	120.9		223.3	*
Error	378:378:378	.020		49.8		38.8	
Age=Late (cycles 5-7)							
Family	16	.126	***	2832	***	680.2	***
reciprocal	1	.013		176.0		27.9	
maternal-1, 18-27	1	.005		178.4		47.6	
maternal-2, 8-7	1	.001		178.4		20.5	
maternal-4, 18-26	1	.001		0.0		6.44	
self vs outcross	1	.416	***	1799	*	1887	***
self-1, 18-27	1	.382	***	118.9		697.3	**
self-2, 8-7	1	.105		6520	***	1416	***
self-4, 18-26	1	.079		112.0		91.4	
Error	357:378:357	.029		366.4		91.2	

significance levels: *** ≤.001, **≤.01, *≤.05

Within early and late ages, family effects were highly significant ($p \leq .01$) for all traits (Table 4). Over the early period an average of 18 propagules were produced per stock plant, and only 13 in the late period. The ranges in family means for number of propagules per stock plant were 13 to 27 early, and 8 to 25 late. Reciprocal effects were generally significant in the early cycles and non-significant late (Table 4). Maternal effects were significant only for parent 1 in the early cycles for number of cuttings and number of propagules. Self effects were significant early and late, but variable by parent. Relative to outcross performance, parent 4 selves were similar, however parent 1 selves produced the most cuttings and parent 2 selves the most propagules.

Combining data over ages shows similar trends (Table 5)-- family effects are highly significant, self effects are significant and variable, while reciprocal and maternal effects are not significant. Overall, an average of 70 usable cuttings per stock plant were produced and 44% rooted. Figure 2 presents the ranges in family and clone-within-family means for all traits. The ranges in family means were 47 to 91 cuttings produced, 32% to 57% rooted, and 19 to 50 propagules produced. Clone-within-family variance was analyzed using binary rooting data (0=not rooted, 1=rooted). Clone variance was highly significant during the early and late periods and over all cycles (data not shown). The ranges in clone means over all families were 12 to 159 cuttings produced, 10% to 82% rooted, and 4 to 95 propagules produced.

Table 5. Analysis of family effects over cycles 2 through 7 on proportion rooted and number of cuttings and number of propagules.

		pRoot		NrCut		NrProp	
Source	df	ms	p>F	ms	p>F	ms	p>F
Family	16	.069	***	4744	***	1449.0	***
reciprocal	1	.030		904.2		549.2	
maternal-1, 18-27	1	.009		892.9		616.1	
maternal-2, 8-7	1	.000		311.3		158.7	
maternal-4, 18-26	1	.011		171.1		170.8	
self vs outcross	1	.346	***	4433	**	4839	***
self-1, 18-27	1	.215	***	26.8		1939	**
self-2, 8-7	1	.129	**	12323	***	5472	***
self-4, 18-26	1	.104	*	465.6		546.2	
Error	378	.018		585.7		178.6	

significance levels: *** ≤.001, **≤.01, *≤.05

Diallel analyses were completed using the fixed model, with reciprocals pooled and without selves. Table 6 gives the results for each trait over all cycles. Both general combining ability (GCA) and specific combining ability (SCA) were non-significant for proportion rooted. For number of cuttings and number of propagules, GCA was significant at $p \leq .01$ and SCA was significant at $p \leq .05$. GCA and SCA effects for each parent and cross are given in Table 7. Clearly parents 1 and 5 were superior in GCA for numbers of cuttings and propagules, while crosses 1 x 3 and 4 x 5 were highest in SCA and cross 1 x 5 was lowest.

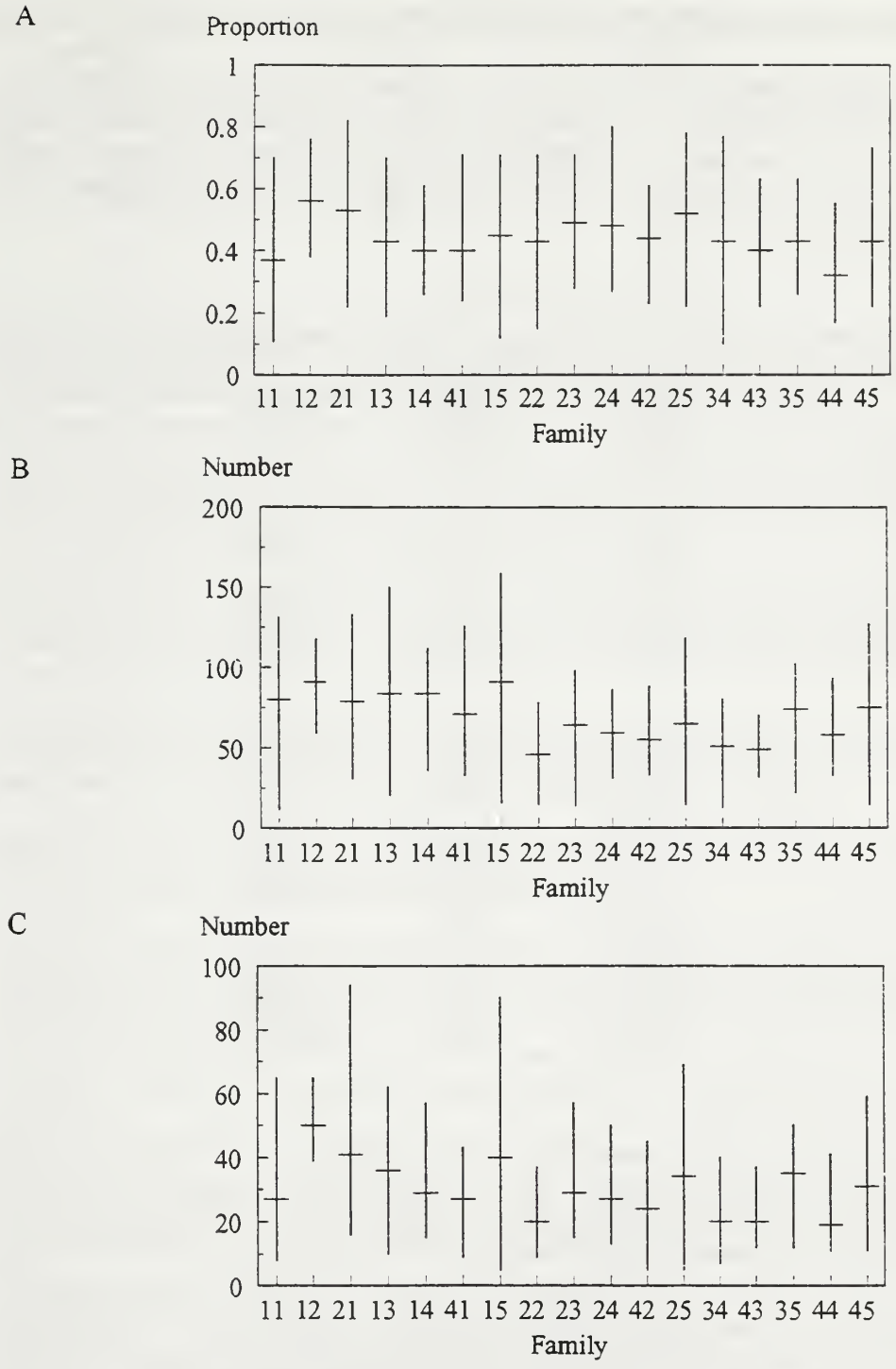

Figure 2. Clone-within-family range for proportion rooted (A), number of cuttings produced (B), and number of propagules produced (C). The bar is plotted from the minimum clone-within-family mean to the maximum clone-within-family mean. The tick mark is plotted at the family mean.

Table 6. Diallel analysis of proportion rooted, number of cuttings and number of propagules.

		pRoot		NrCut		NrProp	
Source	df	ms	F	ms	F	ms	F
GCA	4	0.1194	0.6905	6777	14.48**	2450	12.25**
SCA	5	0.1039	0.6009	1304	2.78*	613	3.76*
Error	315	0.1729		468		200	

significance levels: *≤.05, **≤.01

Table 7. Estimates of general (GCA) and specific (SCA) combining abilities for proportion rooted, number of cuttings and number of propagules.

	pRoot	NrCut	NrProp
General Means	0.480	61.655	25.273
GCA			
parent 1, 18-27	0.005	8.331	3.317
parent 2, 8-7	0.041	-2.174	2.728
parent 3, 9-2	-0.025	-4.715	-3.241
parent 4, 18-26	-0.025	-6.129	-5.152
parent 5, 18-61	0.000	3.779	2.009
SCA			
1 x 2	0.031	1.365	4.884
1 x 3	-0.013	9.329	3.387
1 x 4	-0.013	1.042	-4.209
1 x 5	-0.006	-11.488	-5.099
2 x 3	0.011	2.167	0.474
2 x 4	-0.014	-3.503	-2.506
2 x 5	0.004	-4.147	-2.616
3 x 4	0.009	-7.861	-1.288
3 x 5	0.006	-1.786	-1.448
4 x 5	-0.005	8.061	4.923

The rapid-cycle hedge management system appears to offer promise as a means to efficiently multiply slash pine genotypes. In comparison to loblolly pine (Nelson et al. 1993), slash pine appears to respond better to the rapid-cycle technique. However, improvements in the technique aimed at lessening the mid-season decline are necessary to improve production, especially for the poorer performing families and clones. In addition, serial propagation of the rooted cuttings could be used to further increase productivity (Libby et al. 1974, Wülisch 1984). First season production for the best families and clones were in excess of 40 and 60 rooted cuttings per seedling stock plant, respectively, with an overall average of 32. This level appears sufficient to produce about 500 stock plants per clone in a two year period, which is more than adequate for producing research materials and approaching a level necessary for larger scale applications (Foster et al. 1981).

ACKNOWLEDGMENTS

The authors acknowledge the technical assistance of Tom Caldwell, Jim Hamaker, Gay Flurry, Hershal Loper, and Larry Lott. A copy of the computer software DIALL compiled for use on the PC was kindly provided by Dr. Floyd Bridgewater. Please note that the use of trade names does not imply endorsement by the USDA Forest Service.

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STABILITY OF FUSIFORM RUST RESISTANCE IN LOBLOLLY PINE

Steve McKeand and Bailian Li[1]

Abstract:--In a series of 28 progeny trials across a range of rust infection levels (17% to 74%) in the Gulf and Atlantic Coastal Plains, rust infection was assessed at age 8 years for 43 open-pollinated families. Family means for rust resistance from each test were regressed on the site means to develop prediction equations for rust resistance. The slope of each regression (b) is a measure of stability for each family.

Slopes of the mean family rust level - mean site rust level regressions for 28 of the 43 families did not differ significantly from b=1.0 indicating average stability for 8-year rust resistance for the majority of the families. The highly significant GxE interaction sum of squares from the analysis of variance was contributed by relatively few interacting families. Large variation in R-50 values, ranging from a low of 22% to a high of 75%, indicated substantial variation in fusiform rust resistance in loblolly pine.

Keywords: *Cronartium quercuum*, genetic correlation, genetic variation, heritability, *Pinus taeda*

Introduction

Identification of families of loblolly pine that are resistant to fusiform rust across a broad range of sites is critical to realizing the benefits of breeding programs where rust resistance is important. Most breeding and testing programs assess rust resistance in a relatively small number of tests, and no opportunities exist to assess genetic variation and patterns of resistance across a wide range of rust hazard sites. If important genotype x environment (GxE) interactions exist for rust resistance, either due to differential infection levels or due to differences in rust virulence, testing in relatively few locations under a narrow range of infection levels may not be adequate.

Data from the Good General Combiner (GGC) trials established in the mid-1970's by the members of the N.C. State University-Industry Cooperative Tree Improvement Program were used to study GxE for rust resistance across a wide geographic range in the Gulf Coast and Atlantic Coastal Plains.

[1] Cooperative Tree Improvement Program, Department of Forestry, North Carolina State University, Raleigh, NC 27695-8002.

Material and Methods

Details of the experimental design and measurements are in Li and McKeand (1989). Briefly, each test was a randomized complete block with six replications of 10-tree row plots of 30-50 seedlots. Rust infection was assessed at age 8 years for 43 open-pollinated families established in 28 test in the coastal plain from southeast Louisiana to eastern North Carolina. Each of the 43 families analyzed was in at least 6 tests.

Analyses of variance were conducted on plot means to determine the significance of family and site by family interactions. Heritabilities and genetic correlations were calculated using variance and covariance component estimates from the VARCOMP procedure (REML estimates) in SAS (SAS Institute Inc. 1989).

Rust infection across the tests averaged 39%, ranging from as low as 17% to as high as 74%. Mean rust infection for each family and check seedlot was computed for each test site. The test site mean was computed by averaging all the seedlot means at each site (average of 42 seedlots per site), not just those families that were used for the stability analyses.

To determine the genetic stability of rust resistance, family means from each test were regressed on the site means. The slope of each regression (b) is a measure of stability for each family. Families with a slope not significantly different from b=1.0 have an average stability since their rust infection level directly reflects the average rust infection level of a site. Families with slopes > 1.0 are unstable and are more susceptible than average to increased rust. Families with slopes < 1.0 have high stability and are not as sensitive to increases in rust infection levels.

The predicted rust infection that each family in the GGC trial would have if it had been planted on a rust hazard site of 50% (R-50 value) was estimated through the use of regression equations developed in the stability analysis.

Results and Discussion

Highly significant family effects for rust infection across the 28 tests were found (Table 1). Predicted R-50 values ranged from a low of 22.4% to a high of 75.3%. The wide range of family differences is also demonstrated by the high family-mean heritability of 1.0. The heritability is inflated above what we normally see for rust because of the diverse origin of the families. Provenance differences are confounded with the family differences in this trial.

The family x test interaction was highly significant in the analysis of variance (Table 1) suggesting that GxE for rust resistance could be important. Further analysis of the GxE interaction indicated that GxE for rust resistance is of relatively minor importance. The family x test component of variance was only 3.7% of total variation in the study, while the family variance was 3.6 times larger and contributed 13.5% of the total variance.

Slopes of the mean family rust level regressed on the mean site rust level for 28 of the 43 families did not differ significantly from b=1.0 indicating average stability for 8-year rust

resistance for 2/3 of the families. The relatively high coefficients of determination (r^2) and examination of residuals indicated that the linear models fit the data well. The highly significant GxE interaction sum of squares from the analysis of variance was contributed by about 1/3 interacting families. As previously reported (Li and McKeand 1989), most of the GxE was of minor importance since it was due to heterogeneous regressions and not family rank change at the different sites.

While rust resistance for most families is fairly predictable (average $r^2 = 0.78$), the regression equations for predicting rust resistance are substantially lower than for predicting stem volume in the same tests where average $r^2 = 0.94$ (McKeand et al. 1997). The deviations from regression (e.g. relatively low r^2 values) for the rust regressions suggest more important genotype by environment interactions for rust than for volume. For a family like 11010 (Figure 1) where the $r^2 = .59$, the points that deviate the most from the regression line could be due to 11010's susceptibility or resistance to rust spores of different virulence. No geographic patterns of susceptibility or resistance was evident. For example, 11010 appeared to be very susceptible to rust in the McIntosh, GA test, but in tests in Effingham, GA and Camden, GA the only other tests in that region, the observed rust level was very near the predicted rust level.

The lack of a geographic pattern for differential expression does not preclude the possibility that different virulence strains of fusiform rust exist. Rust virulence variation may not follow any predictable geographic pattern, since we were not able to detect any patterns for resistance or susceptibility.

Deployment Examples

Most families were of average stability and performance (b = 1.0 and intercept = 0), but there were some noted exceptions. About 1/4 of the families were unstable (b > 1.0) for rust resistance. The best example of this type of family is 08061 which would be a poor family to deploy on high rust hazard sites (Figure 1). It's performance is good on low hazard sites (intercept = -10.9) where resistance is not important, but it is very unstable (b = 1.33) and would be susceptible on the high hazard sites.

Table 1. Analysis of variance for % rust infection in the 28 GGC trials at age 8 years.

Source	d.f.	Mean Square [1]	Expected Mean Squares	Variance Comp. (% of Total)
Test	27	23514.74	$\sigma^2 + 6.0\ \sigma^2_{TF} + 13.4\ \sigma^2_{R(T)} + 84.4\ \sigma^2_T$	37.6
Rep(Test)	151	797.57	$\sigma^2 + 23.3\ \sigma^2_{R(T)}$	2.6
Family	42	2632.33	$\sigma^2 + 5.6\ \sigma^2_{TF} + 25.3\ \sigma^2_F$	13.5
Test x Fam	589	516.42	$\sigma^2 + 6.3\ \sigma^2_{TF}$	3.7
Error	3375	334.43	σ^2	42.7
Corr. Total	4184			100.0

[1] All mean squares were highly significant at $P \leq 0.001$.

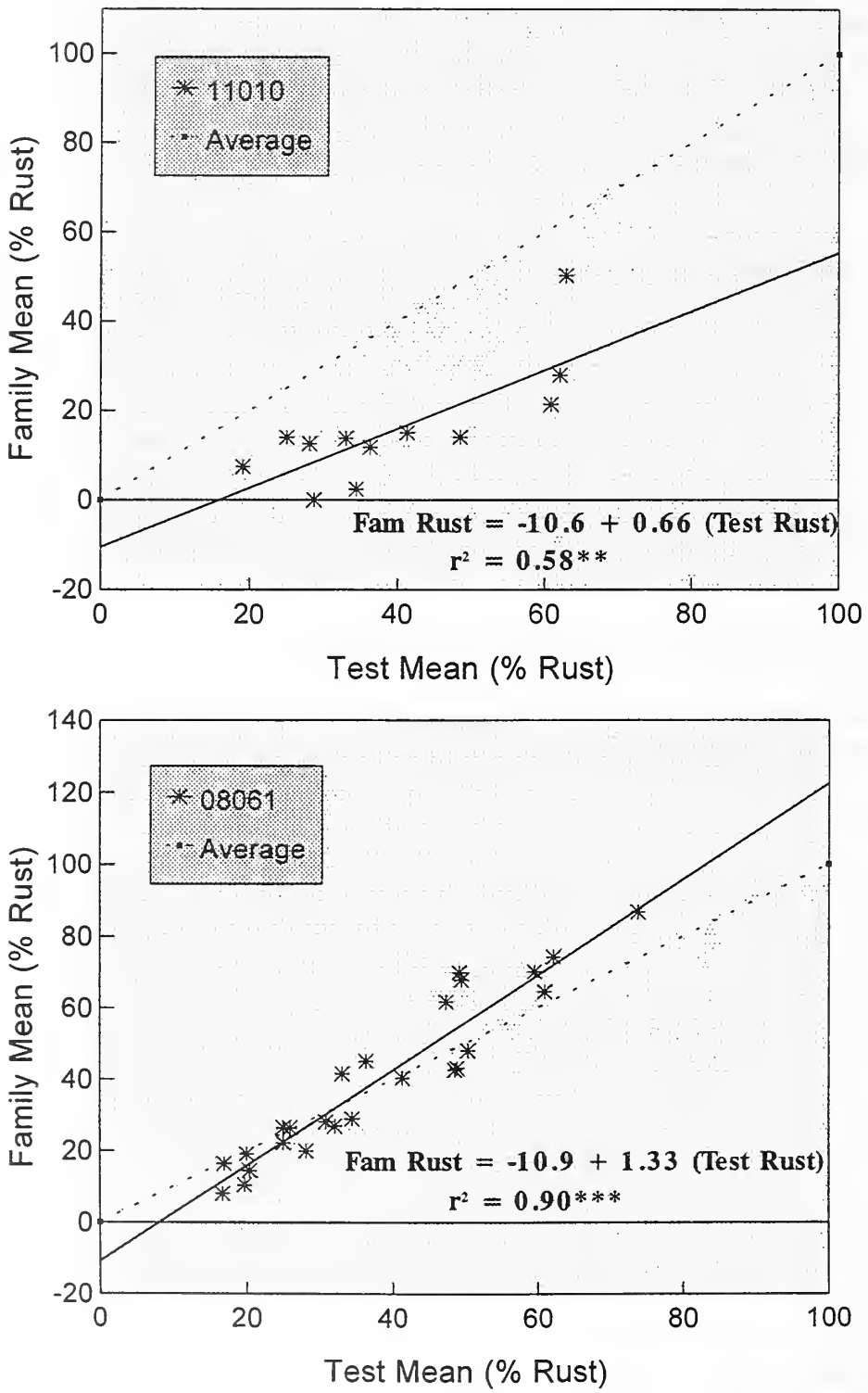

Figure 1. Rust infection for family 11010 (top) and family 08061 (bottom) in the 28 trials used in the stability analyses. (Dashed lines indicate performance of average families).

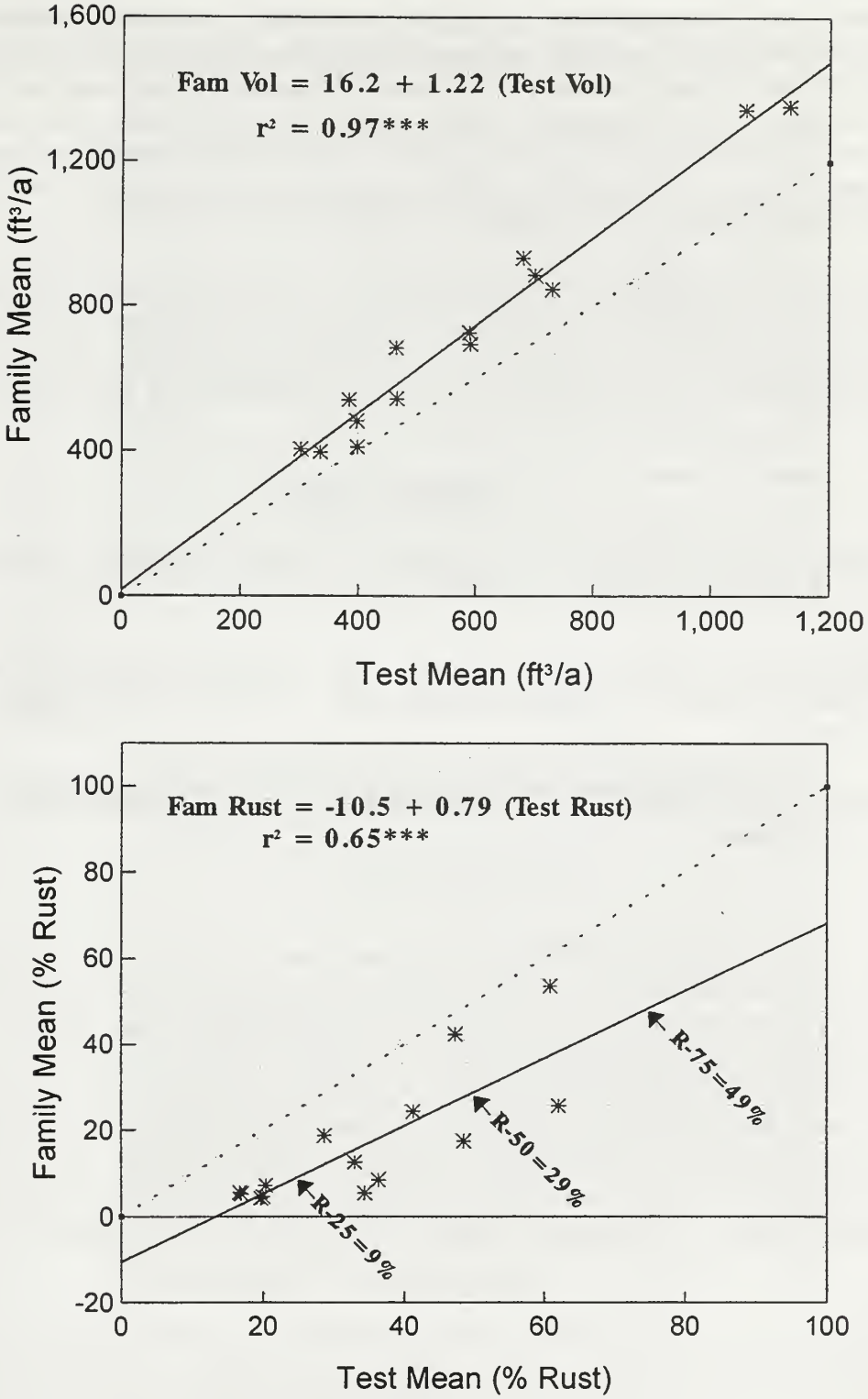

Figure 2. Volume performance (ft^3/acre) for family 11009 (top) and rust performance (bottom) in the 28 trials used in the stability analyses. This is an example of an outstanding family for volume production and rust resistance. (Dashed lines indicate performance of average families).

These rust performance data can be combined with volume predictions from the same trials (McKeand et al. 1997) to guide deployment decisions. Many of these clones are in 1.5-generation seed orchards and are routinely used in regeneration programs in the Gulf Coast and Atlantic Coastal Plain regions. Family 11009 is an excellent example of an exceptional family that combined high volume production (25% above average) and very high rust resistance (Figure 2). This family would be particularly valuable to deploy on the highest rust hazard sites with high predicted volume production.

Although GxE was significant in this population that was tested over a wide range of sites, it should be of relatively little importance in breeding and testing programs. Most of the GxE was due to heterogeneous regressions and not rank change. Testing in relatively few sites with intermediate rust levels should be reliable for predicting rust performance for loblolly pine.

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EARLY GROWTH RESPONSE OF DIVERSE FAMILIES OF LOBLOLLY PINE TO NUTRIENT AMENDMENTS ON A POOR SITE

S.E. McKeand, J.E. Grissom, D.M. O'Malley, and H.L. Allen[1]

Abstract:--Open-pollinated families of loblolly pine (*Pinus taeda* L.) from the "Lost Pines" provenance in Texas and the Atlantic Coastal Plain were established on a droughty, infertile site in the Sandhills of North Carolina where half the trees received nutrient amendments and the other half received no fertilizer. Height growth during the first three years has been evaluated as well as foliar nutrient concentrations. Response to fertilizer applications has been large with a 43% increase in height at age three years. The Atlantic Coastal families were significantly taller than the Texas families in both the fertilized and control plots, and no genotype by environment interactions were observed. While no provenance differences for nutrient concentrations were large, family variation was large, but no genetic association between nutrient concentrations and growth were found. Even given the tendency for low genotype by environment interaction for open-pollinated families of loblolly pine, the adaptability of the Atlantic Coastal families to such extreme environmental conditions was surprising. The long-term performance of the trees will be evaluated to see if this trend continues.

Keywords: Adaptability, height growth, *Pinus taeda*, provenance

INTRODUCTION

Geographic and within-provenance variation for growth and adaptive traits in loblolly pine is very large. General trends in productivity variation are that families from southern and eastern coastal sources grow faster than families from northern, western, and interior sources (e.g. McKeand et al. 1989, Wells 1983, Wells and Lambeth 1983, Schmidtling 1994). Variation in other traits such as fusiform rust resistance (Wells and Wakeley 1966, Wells 1985), stem form (McKeand and Jett 1993, Schmidtling and Clark 1988), and wood density (Belonger et al. 1996) can also be very large. Contrasting the response of two very different provenances of loblolly pine such as from the "Lost Pines" region of Texas and the Atlantic Coastal Plain may give us insight into the adaptive significance of different ecophysiological traits.

Previous work indicates that the Texas sources are generally more stable across environments, while productivity of eastern sources depends more on the environment (van Buijtenen 1978). For eastern sources, productivity was high on the better sites, but very low on the droughty sites. The most important mechanism for drought avoidance may be stomatal control;

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drought-hardy seedlings conserved more water under stress (van Buijtenen et al. 1976), but at the expense of total production (Bongarten and Teskey 1987).

Large genetic differences in productivity among loblolly pine families are being exploited to increase forest productivity in the South. Such productivity differences must result from genetic variation in the ability of trees to acquire and convert solar energy to reduced carbon compounds. The dominant processes determining biomass production of forest stands are light interception, photosynthesis, respiration, and carbon partitioning. Much research has focussed on determining which of these factors has the greatest influence on productivity and how these factors are influenced by environmental stresses (Cannell 1989, Linder 1987). Since light interception and growth efficiency are influenced by soil resource availability, it follows that genetic differences in uptake and utilization of nutrients and water may also contribute to genetic differences in biomass production.

The physiological bases by which genotypes respond to different environments and remain relatively stable in biomass yield are not well understood. Acquisition of resources requires the direct interaction between the genotype and the environment and may explain the relatively high levels of GxE interaction often observed in young stands (Matheson and Cotterill 1990). Results from long-term field trials indicate that for trees at older ages, genotype x environment interactions are less prevalent (McKeand et al. 1990). As full site occupancy is reached, other traits that affect resource utilization and internal recycling of nutrients will become relatively more important. Since these traits involve internal physiological processes, less GxE interaction may be observed.

In this paper, we describe a study designed to assess spatial and temporal variation in response of loblolly pine genotypes to environmental stress. Seedlings have recently completed three growing seasons in the field, and variation in early growth, foliage, and nutritional characteristics are described.

MATERIALS AND METHODS

The study site is located in Scotland County, North Carolina adjacent to the U.S. Forest Service / N.C. State University SETRES (Southeastern Tree Research and Experiment Site) study. The soil is a Wakulla series - sand to greater than 140 feet, sandy, siliceous, thermic Psammentic Hapludult - very infertile, somewhat excessively drained with a total water holding capacity of 7"-8" in a 6.5' profile. The site receives an average annual rainfall of 48". Temperatures average 62°F annually, 79°F summer, and 48°F winter. The existing 10-year-old stand was carefully removed and large block plots of different family-treatment combinations have been established on this droughty, infertile site.

Open-pollinated families from the North Carolina and South Carolina Coastal Plain and from the "Lost-Pines" area of Texas were included in the study. Five families with average or slightly above average breeding values for volume production were used. Seeds were sown in containers (10 in^3 RL Super Cells) in the greenhouse in June 1993, and seedlings were field-planted in November 1993.

To facilitate the application of nutrients, a split-split-plot design was used with the two treatments as main plots, provenances as sub-plots, and families within provenances as sub-sub-plots. The experimental design is:

10	blocks
2	provenances
5	families / provenance
2	treatments (optimal water and nutrition, control)
100	trees/b/p/f/t
20000	total trees

Each plot consists of 100 measurement trees planted at 5'x7' spacing. Buffer trees, 40' around each treatment plot, were planted to eliminate the influence of one treatment on another.

Foliar nutrient ratios (Hockman and Allen 1990) have been used to guide annual fertilizer applications to maintain a balanced supply of all nutrients. Our goal has been to supply optimal levels of nutrients to stimulate rapid growth. Fertilizer additions through the first three growing seasons are shown in Table 1.

Competing vegetation has been controlled in all plots by periodic herbicide treatments and mowing. Likewise, insects (especially tipmoths, *Rhyacionia* sp.) were controlled in the first two growing seasons as needed with periodic insecticide applications.

Table 1. Nutrient additions to fertilizer plots (pounds per acre).

Date	Fertilizer	N	P	K	Ca	Mg	S
June 1994	10-10-10	23.3	10.0	19.3	0	0	<0.4
August 1994	10-10-10	23.3	10.0	19.3	0	0	<0.4
March 1995	12-6-6 + micronutrients[1]	37.3	8.0	15.5	-	-	-
April 1996	Urea + TSP + KMagS	50	5	25	2.5	15	30
	Total	134	33	79	2.5	15	31

[1] Micronutrients included: 0.5 B, 2.0 Cu, 5.0 Fe, 5.0 Mn, 2.0 Zn.

Measurements and Analyses: All trees were measured annually for height and in year 3 for diameter. In January 1997, foliage samples from each plot were taken. Five healthy fascicles from the first flush from the base of the first stem flush from 1996 were sampled from 10 dominant trees in each family plot. After collection, the projected surface area of the foliage (FSA) was measured with an area meter (Delta-T Devices Ltd., Cambridge UK). The needles were then dried for 72 hours at 160°F and average fascicle dry weight (FDW) measured. Specific leaf area (SLA) was calculated as FSA / FDW. After the samples had been ground and sieved through a 1 mm mesh, they were digested using a sulfuric acid-peroxide mix. Foliar nutrient concentrations (N, P, K, Ca, Mg) were estimated for each plot. Analyses for N and P were done colorimetrically using a Lachat QuickChem™ System IV Colorimeter (Lachat Instruments, Milwaukee, WI). Flame emission spectrophotometry was used to determine K, and absorption spectrophotometry for Mg and Ca concentrations using a Perkin-

Elmer 560 atomic absorption spectrophotometer (Perkin-Elmer Corporation). Elemental concentrations were expressed on a percent dry mass basis.

Analyses of variance were conducted on an individual-tree basis for growth data and on a plot-mean basis for nutrient traits. Blocks and families were considered as random effects, treatments and provenances as fixed effects. Correlations of nutrient concentrations and growth traits on family-means basis were estimated rather than genetic correlations because of the limited genetic sample.

Plot means and within plot standard deviations were calculated for height for each 100-tree family plot. Within-plot standard deviations and coefficients of variation were also subjected to analyses of variance to determine if plot uniformity varied.

RESULTS AND DISCUSSION

Survival and growth of the trees has been excellent in the first three years. Survival averaged 93% after three growing seasons (no treatment or genetic effects), and height averaged 6.5 feet. Deer browse and tipmoth caused some problems in the first two growing seasons, and 12.6% of the trees were damaged and not included in the analyses for growth traits.

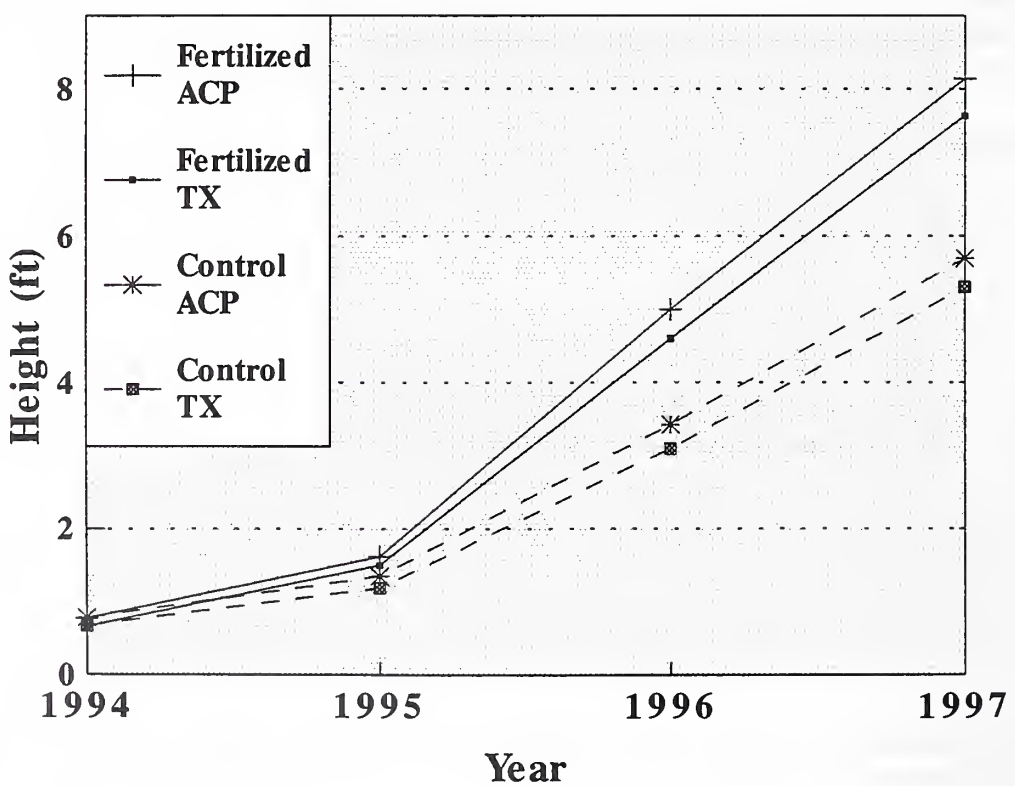

Figure 1. Mean tree heights during the first three growing seasons in the field for trees from the Texas and Atlantic Coastal Plain provenances in the fertilized and control plots. Initial height of seedlings at planting was measured in 1994.

Fertilizer Response. The growth responses to fertilizer amendments were very large and significant each year (Table 2). Height was 23%, 47%, 43% greater in the fertilized plots for years one, two, and three, respectively (Figure 1). Although this is an excessively well-drained site, from the results of the nutrition x irrigation study adjacent to this trial, we know that the primary limit to productivity is nutrition (Albaugh et al. 1997). The huge increase in productivity in the first three growing seasons is possible since all potential nutrient limitations (i.e. more than just N and P) were ameliorated.

One of the most dramatic effects of the nutrition amendment was the increase in uniformity within the 100-tree family plots. The average within-plot coefficient of variation for third-year height was 26.3% for the control plots and 14.3% for the fertilized plots. The within plot standard deviations for height were also significantly different and were 1.43' and 1.12' for the control and fertilized plots, respectively. While increased uniformity typically results from nutritional amendments on very poor sites, the dramatic differences in uniformity was surprising.

Fertilization effects on foliage dry weight and projected surface area were large and significant (Table 2), but they were very small for specific leaf area. For the fertilized plots, average fascicle dry weight was 144 mg and projected surface area was 4.1 cm^2/fascicle. As expected, the foliage in the control plots had significantly less mass (118 mg) and surface area (3.4 cm^2/fascicle). Since foliage production is so critical for stemwood production (Vose and Allen 1988), it is not surprising that the fertilized trees had heavier, larger needles. In future years, we will assess total foliage production on a whole tree basis to better evaluate treatment effects on leaf area - stemwood production.

Foliar nutrient concentrations for nitrogen and phosphorus were not different in the fertilized and control plots (Table 2), even though 134 lbs/a of N and 33 lbs/a of P were applied in the first 3 years (Table 1). We explain this as a dilution effect of having much greater foliage production in the fertilized plots compared to the control plots. Potassium in the fertilized plots (0.38%) was lower than in the control plots (0.47%) even though 79 lbs/a K was applied. Again, dilution effects appear to be the main reason that the faster growing trees had lower concentrations of K. Very little calcium and magnesium was applied in the first three growing seasons (Table 1), so the higher concentrations in the control (Ca: 0.19%, Mg: 0.068%) versus the fertilized plots (Ca: 0.14%, Mg: 0.055%) was also due to dilution.

Table 2. Significance levels for main effects tested in the analyses of variance for growth, foliage, and volume traits at age three years.

Source	Height	DBH	FDW	FSA	SLA	N	P	K	Ca	Mg
Treatment	***	***	***	***				***	***	***
Provenance	*	*						*		
Trt x Prov		*						*	+	
Family (P)	**	*				**	*	**	*	*
Trt x Fam(P)					*					

+, *, **, *** Significant at P≤0.10, 0.05, 0.01, 0.001, respectively.

Provenance and Family Variation. As expected, the five families from the Atlantic Coastal Plain grew faster than the five Texas families (Figure 1). We anticipated that under the harsher environmental conditions in the control plots that the Texas families would perform relatively better. However, the ACP families were superior in both environments, and the provenance by treatment interactions for height in all three years were not close to being significant. Although there was a provenance by treatment interaction for DBH (Table 2), there was no provenance rank change in the two environments, only a difference in the magnitude of the differences (greater in the fertilized plots).

Families within provenances also differed for growth traits (Table 2). The family means for the ACP families varied from 5.2' to 5.8' in the control plots and from 8.6' to 10.2' in the fertilized plots. The Texas families also differed in the control plots (4.7' to 5.0') and in the fertilized plots (7.8' to 8.6'). The marked difference in productivity between the drought-hardy Lost Pines families and the ACP families is illustrated by the lack of overlap of the family means for height.

The lack of genotype by environment interaction both at the provenance and family level was surprising. Given the magnitude of the imposed environmental differences and the young age of the trees, differential performance of the families in the two treatments were expected. This result reinforces the tenet of the stability of open-pollinated families of loblolly pine.

In general, provenance differences were minimal for foliage traits and nutrient concentrations. Only for potassium, did the two provenances differ (TX: 0.41%, ACP: 0.44%). The ACP had higher concentrations for K in both treatments, despite the significant provenance by treatment interaction; only the magnitude of the differences changed.

Family differences within provenance were significant for all foliar nutrient concentrations but not for FDW, FSA, and SLA. Genetic variation in nutrient concentrations is common in loblolly pine (e.g. Li et al. 1991, Vasquez 1993), but the association with productivity variation has been poor (McKeand and Svensson 1997). In the present study, family differences in foliar nutrition were not correlated with any growth or foliage trait (data not shown).

Future Work. This experiment will be a long-term (~20 years) field laboratory for ecologists, physiologists, and geneticists to study the bases for trees' responses to environmental stress. Future work will emphasize both above- and below-ground production and physiological processes and how they interact to affect productivity. Not only will traditional quantitative genetic analyses be conducted to evaluate genetic control for these traits, but genomic mapping to determine the significance of major gene control is also an integral part of the study. Megagametophytes for each of the 20,000 individuals in the trial are in cold storage (-80°C) and DNA will be extracted and genomic maps developed to determine marker - trait associations. Using the open-pollinated families in such a manner will allow us to determine if major genes with high breeding value (O'Malley and McKeand 1994) are associated with adaptive response to environmental stress.

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VARIATION AMONG OPEN-POLLINATED FAMILIES OF *PINUS CARIBAEA* VAR *HONDURENSIS* FROM GUANAJA ISLAND, HONDURAS, GROWN IN BRAZIL.

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W. S. Dvorak[2]

Abstract: *Pinus caribaea* var. *hondurensis* (Sénécl) Barr. & Golf. is a tropical pine that naturally occurs in lowland areas of Belize, El Salvador, Guatemala, Honduras Nicaragua and eastern Mexico. Over the last 20 years it has been one of the most studied tropical pines and the one with the most commercial importance in central and northern Brazil. One of the most productive *P. caribaea* provenances in Brazil is Guanaja Island that is located 60 km off the northern coast of Honduras. Thirty-nine open-pollinated families from Guanaja Island were grown in a progeny test in the Brazilian "Cerrado" (tropical savanna) at 15° 35' S latitude and 1000 m altitude, and assessed at ten years of age. The traits of height, diameter (dbh), volume, branch diameter, stem straightness and multistem appeared to be under moderate additive genetic control with individual tree heritability of 0.28, 0.30, 0.31, 0.10, 0.24 and 0.13 respectively. If the best 20 trees for volume are selected in the trial regardless of quality, the estimated genetic gain in the next generation would be approximately 30%. Because the tree stem form and branching patterns of unimproved *P. caribaea* from Central America and Mexico are often poor, selection criteria for quality traits may need to be relaxed in the first generation of breeding in order to make large genetic gains in productivity.

Keyword: pine, breeding, genetic gain, heritability

INTRODUCTION

Pinus caribaea var. *hondurensis* (Sénécl) Barr. & Golf. is a tropical pine that occurs in lowland areas of Belize, El Salvador, Guatemala, Honduras, Nicaragua and in one location in the state of Quintana Roo, Mexico. The species has great commercial importance in places like Queensland, Australia, central and northern Brazil, the Fiji Islands, and Venezuela. The largest plantation area of *P. caribaea* is in Venezuela where

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approximately 600,000 hectares have been established and 30,000 hectares are planted annually.

In the 1970s, a number of *P. caribaea* provenances were tested throughout the tropics under the auspices of the Oxford Forestry Instiute (OFI). One of the most promising *P. caribaea* populations was Guanaja Island, Honduras, located approximately 60 km north of the mainland of the country. The stands on the island are phenotypically poor having been high graded by the local populace for several decades and are subjected to annual fires set by cattle ranchers. However, trees from Guanaja Island demonstrated good growth and high wood density relative to some other Caribbean pine populations in the international provenance trials.

Provenance/progeny tests of *Pinus caribaea* were subsequentely established in a second phase seed collection program by both the OFI and the Central America and Mexico Coniferous Resources Cooperative (CAMCORE), North Carolina State University in the early 1980s (Dvorak and Donahue 1992). The objectives for the second set of trials were to better determine family and within family variation and establish *ex situ* conservation plantings. CAMCORE made collections in 24 provenances and sampled well over 1000 mother trees in Mexico and Central America, including 75 trees from Guanaja Island (Dvorak and Donahue 1992). The Guanaja Island collections were planted across multiple sites in Brazil, Colombia, and Venezuela.

One trial of the CAMCORE Guanaja material was established at Planaltina, Brazil, located at 15° 35'S and 1000 m altitude in the "Cerrado" region of the country. This paper reports on the growth, heritability, and expected genetic gain from selection in the Guanaja material at Planaltina and discusses the performance of similar material planted in other countries in northern South America.

MATERIAL AND METHODS

Seed were collected from 75 mother trees of *P. caribaea* var. *hondurensis* on Guanaja Island in 1983 by the CAMCORE Cooperative and the National School of Forest Science (ESNACIFOR) in Siguatepeque, Honduras. The selected trees were of average to excellent phenotypic quality and were separated by a distance of 100 m whenever possible following CAMCORE guidelines (Balocchi 1990). The island of Guanaja has gently rolling to hilly topography and trees were sampled at altitudes between 65 and 165 m. The phenotypically best trees were found in valleys that were protected against seasonally strong winds and where the soils were most fertile. The average annual rainfall on Guanaja is approximately 2500 mm, most of which occurs from October to January.

Seeds from the collection were sent to CAMCORE organizations in Brazil, Colombia, and Venezuela. One trial in Brazil was established by EMBRAPA at Planatina. Thirty nine open-pollinated families were included in the test. These were established at 3 m x 3 m spacing in a unbalanced single tree plot design with 22 replications. The number of trees in each family varied from 6 to 22, due to uneven number families in replications and also due to mortality during the life of the trial.

The trial was assessed at 10 years of age for survival, height, dbh, volume, stem form, branch diameter, broken tops, forking, foxtails, multistems, and presence of cones. Both branch diameter and stem form were scored using a subjective scale of 1-3, with 1 being the largest or most crooked and 3 being the smallest or straightest (Balocchi 1990). Broken main stems from wind damage, forking, foxtails, cone presence and multistems were scored either as a "yes" or a "no". A tree was classified as multistem when it had two or more stems coming from the base. Volume was calculated from a general formula for juvenile trees derived by Ladrach (1986) as volume (m^3) = 0.00003 d^2h.

The data was analysed using the GLM procedure of SAS and the RANDOM option to accommodate the unbalance in the design. Due to the difference in the number of families within each replication, the residual degrees of freedom used in the F-test varied and were estimated using Satterwaitte's (1946) approximation. The VARCOMP procedure in SAS was used to estimate variance components. Individual and family heritability was estimated in the normal way with the exception that the coefficient of relationship was assumed to be 0.33 instead of 0.25 for half-sib analysis because 1) there was a high probability that the Caribbean pine trees at Guanaja were inbred to some extent, and 2) it is likely that the open-pollinated families are not truly half-sib, but contain some full-sibs.

The data for foxtails, forking, fruiting, and survival were not analyzed for this study because only single tree plots were used. Instead, family means are presented.

Expected gain (EG) was calculated using the expression below:

$EG = h^2_i \times SD_i$

where:

EG = Expected Gain for individuals;

h^2_i = individual tree heritability;

SD_i = Selection differential for individual.

RESULTS

The study at Planaltina, Brazil

Survival of Caribbean pine trees at Planaltina was 95% after 10 years of age. Family differences were significant for all traits analyzed (Table 1). The average height growth for the Guanaja material was 16.1 m and the mean difference between the best and the worst family in the test was 3.5 m at ten years of age. The individual tree volume of the best family was 59% greater than the worst family. Individual tree heritability for growth traits ranged from 0.28 to 0.31 and family heritability varied from 0.68 to 0.71 (Table 1).

The stem form of the Guanaja trees at Planaltina was poor and had an average score of 1.2. The best family for stem form in the trial had 14% of its trees classified as "3", *i.e*, perfectly straight. There were several families that had mostly small, thin branches (Table 3). Individual tree heritability for stem form and branch diameter was 0.24 and 0.10, respectively (Table 1).

The frequency of forking (11%), foxtails (2%), and broken tops (1%) was low in this trial. Cone production at 10 years of age was also low with only 0.1% of the trees having cones. However the frequency of multistem trees in the test was high (24.8 %) with some family means as high as 40% (Table 3).

Ninety percent of the trees in the tests had some kind of defect (poor stem form, branchness, multistem etc.). When all the defected trees were removed from the analysis and the best remaining 20 selected, the estimated genetic gain for volume was 12%.

Performance of Guanaja material in other locations

Three other tests of Guanaja material were established at El Hierro, Venezuela (lat. 9° 10'N, and altitude 150 m), El Amparo, Colombia (lat. 9° 45'N, and altitude 100 m) and Jari, Brazil (lat. 0° 54' S, and altitude 200 m) with as many as 28 open-pollinated families in common to the ones planted at Planaltina. Growth rates varied considerably in these trials depending on the planting site as did forking and foxtail percents (Table 2). For example, the growth rate at El Amparo, Colombia, was substantially poorer than the other three sites; foxtail percents at Jari and El Hierro were 20% and 40%, respectively, versus 2% at Planaltina. Furthermore, family ranks for volume changed considerably across location and genotype x site interactions appeared to be of practical significance (Table 4).

DISCUSSION

The Guanaja material at Planaltina has proven to have excellent growth and vigor; not only has this provenance grown well compared to other *P. caribaea* var. *hondurensis* populations but is also superior to *P. oocarpa* and *P. tecunumanii*. Heights of Guanaja Island trees at 10 years of age are slightly less than the ones reported for provenances of Mountain Pine Ridge, Belize; Poptun, Guatemala and Culmi, Honduras at 12 years of age grown in central Brazil (Moura *et al.* 1991). Average height growth of Guanaja *P. caribaea* at Planaltina was also superior to Guanaja material growing in New Caledonia after 14 years (Cremiere 1989). At Cardwell, Queensland, Australia, Guanaja was significantly more productive than all the other Caribbean pine provenances tested (Kanowski *et al.* 1989) and also was the case at Jocón Honduras. However the Guanaja Island source performed less well at Carta and poorly at Culmi, Honduras (Cornelius and Ponce 1989). The poor stem form of the Guanaja material has also been reported elsewhere. In New Caledonia, the insular material of Guanaja had good vigor but poor stem form making selection difficult, when compared to other coastal provenances (Cremiere 1989). However at Cardwell, Australia, Guanaja and Alamikamba were the straightest provenances (Kanowski *et al.* 1989). Environment apparently has great effect on stem form.

The environmental conditions in the Brazilian cerrados are not only favorable to vigor of Caribbean pine but also to low rate of mortality, foxtail and forking, in contrast to other sites in Brazil, Venezuela and Colombia. (Dvorak & Donahue, 1992; Vásquez & Dvorak 1996). Cremiere (1989) reported that Guanaja had poorer resistance to wind than the coastal provenances of *Pinus caribaea* var. *hondurensis* in New Caledonia. At Planaltina, wind damage has not been of any importance.

Pinus caribaea does not produce good cone crops in the region of the cerrados compared to other pine species like *P. oocarpa* (Moura *et al.* 1996). Under other environmental conditions *P. caribaea* var. *hondurensis* starts fruiting at four to five years in Vietnam (Kha *et al.* 1989). Survival is thought to be better at Planaltina than in the western llanos of Venezuela and eastern Colombia because the soils are deep, have high clay contents and are not compacted. High variability was found in all growth and quality characteristics. Family, and individual h^2 was high indicating that good gains can be achieved in the selection process (Table 1).

Selecting the best 20 individuals for height, dbh and volume regardless of quality, would result in expected genetic gains in the order of 6.1%, 30.9% and 31.1%, respectively, at Planaltina. However, when stem form, branching and other defected

characteristics are considered these gains for height, dbh and volume are reduced to 3.7, 4.2 and 11.9%. If the defected trees are excluded from the selection process, only 73 out of 804 would be available in the breeding population. In populations of *P. caribaea* var. *hondurensis* at other sites, a high proportion of defected trees was also observed. Even moderate selection for quality would eliminate most of the trees considered as good candidates for volume in the next cycle of breeding. To maintain a reasonable selection differential for the volume, the selection criteria should be relaxed for quality traits, during the first cycle of selection.

The selection and use of this important material of *P. caribaea* from Guanaja is extremely important for the development of pine forestry in the cerrado region of Brazil. The cerrados has an area of 204 millions ha and less than 2% are occupied by forest plantations. Because the use of the native trees species are restricted and the high demand for sawn timber in the region both pine and eucalypt plantations will be extremely important in the future.

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Table 1. Heritabilities for *Pinus caribaea* var. *hondurensis* provenance of Guanaja Island, in Planaltina-DF, Brazil at 10 years.

Characteristic	Mean	CV(%)	Heritability	
			h^2_f	h^2_i
Height	16.0 m	10.7	0.70	0.28
dbh	0.18 m	18.3	0.71	0.30
volume	0.26 m^3	43.3	0.68	0.31
Branch diameter	1.9	39.0	0.42	0.10
Stem form	1.2	32.4	0.65	0.24
multistem	24.9%	43.6	0.49	0.13

Table 2. Means of different traits at age five years for families of *Pinus caribaea* var. *hondurensis* from Guanaja Island, grown at Planaltina and Jari, Brazil; El Hierro, Venezuela and El Amparo, Colombia.

Characteristic	Planaltina Brazil	El Hierro Venezuela	El Amparo Colombia	Jari Brazil
Height (m)	7.8	7.1	4.8	7.4
dbh (m)	0.13	0.13	0.09	0.12
volume (m^3)	0.04	.0.04	0.02	0.04
fox tail	1.31	41.6	-	21.8
forking	2.8	27.8	-	26.6
survival (%)	96.7	70.9	82.0	96.0

Table 3. Family means and percentage of height (H), dbh, volume (vol.), stem form (ST), multistem (MS), forking (FK), foxtail (FX), fruiting (FR) and broken-top (BT) of the Guanaja Island provenance of *P. caribaea* var. *hondurensis* at Planaltina-DF, Brazil.

FAM	H (m)	dbh (cm)	vol. (m^3)	ST	BD	MS (%)	FK (%)	FX (%)	FR (%)	BT (%)
326	16.54	20.11	0.30	1.09	1.70	11.1	3.7	0.0	0.0	0.0
327	16.06	17.73	0.25	1.09	1.66	37.2	2.9	0.0	0.0	2.9
332	15.16	17.77	0.19	1.17	1.95	42.9	9.5	0.0	0.0	0.0
336	14.58	18.33	0.26	1.00	1.50	16.7	0.0	16.7	0.0	0.0
337	16.38	18.52	0.31	1.12	1.88	22.3	5.6	0.0	0.0	0.0
338	15.33	16.83	0.20	1.18	1.74	28.6	4.8	9.5	0.0	0.0
339	16.03	17.48	0.22	1.04	1.04	17.2	0.0	3.4	0.0	0.0
341	16.32	19.00	0.31	1.02	1.57	7.1	0.0	0.0	0.0	0.0
343	13.68	16.72	0.22	1.21	1.93	7.6	0.0	0.0	0.0	0.0
345	15.55	19.13	0.26	1.22	1.75	34.7	0.0	0.0	0.0	0.0
347	16.40	17.65	0.24	1.24	1.52	14.3	4.8	4.8	0.0	0.0
348	16.50	19.61	0.32	1.10	1.85	35	0.0	0.0	0.0	0.0
350	15.37	16.38	0.17	1.20	1.77	5.9	5.9	5.9	0.0	0.0
351	16.90	19.68	0.29	1.33	1.93	0.0	0.0	0.0	0.0	0.0
352	16.75	19.45	0.30	1.15	1.84	16.7	5.6	11.1	0.0	0.0
353	16.50	19.28	0.29	1.09	1.59	20.6	3.4	3.4	0.0	0.0
354	15.96	17.53	0.23	1.36	2.07	71.4	0.0	0.0	0.0	0.0
355	16.61	18.43	0.28	1.23	2.02	29.6	0.0	3.7	0.0	0.0
356	17.11	19.37	0.29	1.08	1.85	7.7	0.0	7.7	0.0	0.0
358	15.34	18.03	0.25	1.62	1.81	12.5	0.0	0.0	0.0	0.0
359	16.34	18.35	0.26	1.05	1.79	31.6	0.0	0.0	0.0	0.0
360	16.00	17.38	0.24	1.32	2.04	32.8	4.6	0.0	0.0	4.5
363	16.06	18.18	0.27	1.06	2.17	27.8	5.6	0	0.0	0.0
365	15.76	18.03	0.26	1.05	1.90	14.3	9.5	0.0	0.0	0.0
366	16.45	19.07	0.26	1.42	2.00	31.6	5.3	0.0	0.0	5.3
370	15.58	18.69	0.24	1.17	1.78	50.0	11.1	0.0	0.0	0.0
373	16.15	18.10	0.22	1.23	1.69	11.5	3.8	11.5	0.0	7.7
378	17.00	17.79	0.27	1.20	2.20	40.0	0.0	10.0	0.0	0.0
379	16.16	17.75	0.23	1.05	2.10	31.6	0.0	0.0	0.0	0.0
381	15.77	18.53	0.26	1.08	2.08	23.8	0.0	0.0	0.0	0.0
382	16.09	17.70	0.24	1.19	2.19	43.7	0.0	0.0	0.0	0.0
383	16.29	18.94	0.32	1.08	1.38	12.5	0.0	6.3	0.0	6.3
384	17.05	18.69	0.28	1.37	2.17	37.5	6.3	0.0	0.0	0.0
387	16.15	17.42	0.23	1.05	1.85	25.9	7.4	7.4	0.0	0.0
388	14.63	13.90	0.19	1.40	2.40	22.2	0.0	5.6	0.0	5.6
390	16.09	16.91	0.21	1.13	1.90	17.2	6.9	3.4	0.0	0.0
391	16.80	22.31	0.41	1.40	1.55	17.9	3.6	0.0	0.0	0.0
397	16.25	18.79	0.28	1.41	2.05	19.2	0.0	0.0	3.8	0.0
398	15.91	17.46	0.24	1..43	2.24	39.4	0.0	0.0	0.0	0.0
mean	16.04	18.23	0.26	1.19	1.86	24.9	2.8	2.8	0.1	0.8

Table 4 Family volume means (m^3) of *P. caribaea* var. *hondurensis* from Guanaja Island, growing at different sites and with different ages.

Family	Planaltina (10 years)	El Amparo (5 years)	El Hierro (8 years)
391	.412	-	.172
383	.322	.014	.167
338	.199	.015	
353	.290	.015	-
351	.295	-	.205
352	.304	.015	-
373	.219	.011	-
347	.239	.016	-
348	.324	.020	-
359	.262	.028	-
397	.284	-	.162
384	.284	-	.157
366	.257	.017	.174
354	.233	.016	-
360	.236	.013	.167
337	.310	.012	.187
387	.226	.020	.170
345	.262	-	.179
363	.271	.019	-
365	.261	.013	-
356	.295	.019	-
358	.248	.012	-
327	.248	.020	-
339	.225	.018	-
378	.271	.016	.164
381	.262	.022	.161
390	.208	.010	-
355	.281	.017	.172
370	.243	.011	-
350	.172	.013	-
379	.235	.013	.174
382	.242	013	-
398	.240	-	.161
388	.187	.013	.172

TREE IMPROVEMENT AND REPRODUCTIVE BIOLOGY STUDIES IN TAMARIND

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Abstract:-- Reproductive biology and breeding system were studied in five Tamarind clones. Considerable phenological variations were observed between clones. Flowers showed entomophilous adaptations, open pollination fruit setting was 1 - 2 %. Controlled pollinations indicated that it is a preferential outcrosser with very little selfing, apomixis was absent. Pollens showed dimorphism and low sterility, long term storage was possible. Fruits were produced in pink and green colours.

Key words: Breeding system, Clone, Controlled pollination, Dimorphism, Phenology, Pollen storage, Tamarind.

INTRODUCTION

Tamarindus indica L. commonly known as tamarind is a monotypic genus belonging to the family Leguminosae and is widely distributed in Africa and Asia. It grows upto forty five feet in height and has a dense spreading crown with a clear trunk and grows in a wide range of agroclimatic conditions and is a highly drought tolerant species . It is an excellent multipurpose tree species which is used as food, food preservatives (Tsuda 1995), fodder (Kaitho 1996), drugs (Mustapha 1996), timber and fire wood. Tamarind fruit pulp is very rich in ascorbic and tartaric acids and it is the most commonly used preservative in pickle industry.

Tree improvement activities in tamarind were initiated in India almost a decade ago. Many forest agencies have surveyed and identified high yielding genotypes and have also established germplasm banks. Generally tamarind plantations are raised from seedlings, but nowadays tamarind clonal planting is also becomming popular. Though tamarind is mainly grown for fruits, it has been very poorly understood for its reproduction. Hence a detailed study was carried out with the following objectives :

* to know the phenology and floral biology
* to understand the breeding system

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MATERIALS AND METHODS

Phenology recording. Studies were conducted during 1996 in five tamarind clones NBN1, NBN2, NBN3, RDB Patna and JRK (herein after refered as C1,C2,C3,C4 and C5) in a State forest department clonal bank at Karnataka in India. In each clone five ramets were selected for observation. Phenological parameters such as terminal and axillary shoot elongation,leaf production, inflorescence length and flower production per inflorescence and per branch were recorded. Five hundred (hundred per ramet) measurements or counts were made depending upon the characteristic.

Controlled pollination. Controlled pollination was done in four clones (C1,C2, C4 and C5) using a full diallel mating design (Zobel and Talbert 1984). Sixteen crossing and selfing combinations and four apomixis treatments were made. Flowers were emasculated with aid of a clean fine tip forceps from 15.00 to 20.00 hrs and flowers were pollen dusted using a dry painting brush or needle from 6.00 to 11.00 hrs. In an inflorescence only the treated flower was retained. In each clone 100 flowers were operated per day per treatment and all treatments were repeated for seven days. Thus 700 flowers were operated for each treatment. Flowers operated were caged in paper covers (in size of 12 x 7 cms). and were tagged properly. Bags were removed on the seventh day for recording fruit set.

Pollen biology. Pollen samples were stored in clean 1.5 cm. diameter plastic Petri plates in ambient (37°C) and cold (5°C) conditions. Prior to cold storage the pollen was dried in sunlight for 2 - 3 hours in the mornings. Pollen viability was assesed using a differential stain, in which viable pollen stained pink and dead ones stained green (Alexander 1969). Slides were prepared and analysed according to the procedures described by Radford etal., (1974).

Data Analysis. ANOVA, T - Test (Waller and Duncan 1969) and coeffiecient of correlations of means were done using SAS package 6.09E version.

RESULTS

Floral biology. Flowers are showy, bisexual, herkogamous, five sepals, five petals, three anthers fused at base with filaments incurving towards the ovary base, style simple, stigma is unbranched and pappillate, ovary superior with 12 - 14 ovules. Ovary base has numerous hairs with copius nectar. Pollination is mostly by honey bees. Anthesis starts at 20.00 hrs and flowers are completely unwound by 02.00 hrs, but anther dehiscence is only by 08.30 in the morning. Stigma is receptive for almost 48 hours with peak receptivity on the day of anthesis. Fruits are produced in two distinct colours,clone C2 produced pink fruits while all other clones produced green fruits.

Vegetative phenology. Vegetative shoots are produced annually and they bear flowers only the next season. Two types of terminal shoot production could be observed, Clones C1 and C2 produced shorter terminal shoots ("erect type") C3, C4 and C5 produced long shoots ("drooper type") (Table 1). Clones also varied considerably for axillary shoot length. Clones with lengthy terminal shoots produced more foliage, clone C5 showed the maximum foliage production (Table 1).

Reproductive phenology. Production of flowers varied between clones (Table.1). Clones with longer vegetative terminal shoots produced more flowers (Table.1). Ovary and style length varied between clones (Table.1).

Table.1. Vegetative and reproductive phenology in tamarind clones

	Clones							
	C1	C2	C3	C4	C5	Sem	LSD at 5%	CV%
Terminal shoot length(cm)	15.42_{D}	14.38_{E}	19.12_{C}	19.41_{B}	21.45_{A}	0.039	0.084	0.347
Axillary shoot length(cm)	6.75_{D}	6.18_{E}	8.41_{B}	7.54_{C}	9.34_{A}	0.008	0.017	0.167
Terminal shoot leaf length(cm)	8.24_{C}	8.24_{C}	8.15_{D}	9.37_{A}	9.12_{B}	0.021	0.043	0.376
Leaves per terminal shoot	8.78_{D}	8.55_{E}	13.76_{A}	12.18_{C}	13.13_{B}	0.016	0.034	0.224
Leaves per axillary shoot	5.34_{A}	4.45_{B}	4.06_{C}	4.05_{C}	3.67_{D}	0.067	0.14	2.45
Inflorescence length (cm)	4.38_{D}	4.85_{C}	5.70_{A}	5.07_{B}	4.91_{B}	0.110	0.234	3.50
Inflorescence per branch	7.56_{D}	13.69_{B}	13.59_{B}	16.32_{A}	11.92_{C}	0.174	0.368	2.18
Flowers per inflorescence	13.28_{C}	8.05_{E}	15.57_{B}	17.09_{A}	12.21_{D}	0.024	0.053	0.296
Flowers per branch	100.47_{E}	109.12_{D}	212.70_{B}	279.80_{A}	145.61_{C}	2.769	5.871	2.583
Style length(mm)	4.57_{B}	4.62_{A}	4.74_{A}	4.30_{C}	4.64_{A}	0.064	0.137	2.237
Ovary length(mm)	6.60_{B}	6.74_{A}	6.61_{B}	6.13_{D}	6.21_{C}	0.038	0.080	0.927

Means with same letters are not significantly different by Duncan's Multiple Range test (p=0.05)

Pollen biology. Pollen sterility was found to be very low (Table 2). Under ambient conditions (37°C - 40°C) pollen viability was nearly 88% until 3 days. Pollen stored in 4 ℃ remained viable upto 97% till 100 days. Pollens were produced in two distinct sizes (40 uM and 25uM), pollen dimorphism percentage increased in the late flowers (Table 2).

Table 2. Pollen biology of Tamarind

	Clones					Sem	LSD at5%	CV%
	C1	C2	C3	C4	C5			
Pollen sterility (%)	1.18_B (1.08)	1.13_B (1.06)	0.79_C (0.88)	1.80_A (1.33)	1.93_A (1.38)	0.038	0.081	5.262
Pollen dimorphism(%)	11.03_C (3.32)	13.39_A (3.65)	12.08_B (3.47)	9.63_E (3.10)	10.23_D (3.19)	0.039	0.083	1.856
Pollen viability in ambient storage (%)	88.00_A (9.38)	88.20_A (9.39)	84.80_A (9.20)	85.60_A (9.25)	86.60_A (9.30)	0.145	NS	2.464
Pollen viability in cold storage (%)	97.40_A (9.87)	96.80_A (9.84)	97.0_A (9.85)	96.60_A (9.83)	97.0_A (9.85)	0.032	NS	0.523

Means with the same subscript are not significantly different by Duncan's Multiple Range test (p=0.05). The values in parenthesis are transformed means (square root transformation)

Breeding system. Fruit set under open pollination condition ranged 1 to 2% among the clones, Clone C2 showed significantly higher fruit set than other clones (Table 3). In controlled cross pollination fruit set was as high upto 88% in clone C1, in contrast only very low fruit setting was observed in selfing (Table 3). Clone C2 showed highest rate of selfing than others. Cross incompatibility and apomixis were absent.

Table 3. Breeding system of Tamarind

	Clones					SEm	LSD at5%	CV%
	C1	C2	C3	C4	C5			
Open pollination (%)	1.65_B (1.28)	2.32_A (1.51)	1.15_C (1.07)	1.47_B (1.21)	1.44_B (1.19)	0.082	0.174	10.379
Cross pollination (%)	84.20_A (9.16)	88.20_A (9.39)	87.40_A (9.34)	75.80_B (8.70)	na	0.182	0.396	3.145
Self pollination (%)	2.40_C (1.51)	6.80_C (2.59)	2.60_C (1.59)	4.60_B (2.13)	na	0.107	0.234	8.683

Means with the same letter are not significantly different by Duncan's Multiple Range test (p=0.05). Values in parenthesis are transformed means (square root transformation) parenthesis.

DISCUSSION

Zobel and Talbert (1984) have opined that knowing the biology of tropical trees is critical before initialising any tree improvement programme. Knowledge on reproduction is one important aspect which needs much attention, also it helps to know the amount of genetic variation in a species (Costich 1995). In tropical trees many reproductive biology studies have been made on ecology and evolutionary terms (Bawa etal., 1985) however only a very few

applied studies are available (Venketesh and Sharma 1975, Egenti 1976, Veerendra and Ananthapadhmanaba 1996).

In this study we were able to understand the patterns in vegetative phenology and their considerable influence over reproduction. Clones with longer vegetative terminals shoots clearly showed higher flower production. Long inflorescences are invariably more attractive to insects (Inoue 1985) and often have a greater probability of maturing in to fruits (Ackerman and Montalvo 1985) selection of clones with longer inflorescences should be advantageous while raising clonal plantations.

Very low fruit set in open pollination (1-2%) in tamarind is not an unusual phenomenon, such a low flower to fruit ratio is known in many tropical tree species (Nagarajan etal., 1996). These failures are because of pollinator limitation (Calvo 1990),inadequate visits of pollinators (Aker and Udovic 1981) or may be due to self-incompatibility which is quite common in tropical trees (Bawa 1974, Kaur et al., 1977, Chan 1981). Also in legumes a tripping mechanism is known to exist, in which in a mixed pollen dusting flowers prefer cross pollen against self pollen (Arroyo 1978). This process considerably influences fruit setting in open pollination.

Though plants are known to show increased fruit set with cross pollen (Johnson 1991, Young and Young 1992), studies made earlier in tropical trees have reported only low fruit settings (Egenti 1976) due to the pollination techniques used, flower abortions (Bawa and Webb 1984), and inbreeding (Haber and Frankie 1982). In this case high fruit setting was because only the earliest formed flower was used for crossing. It is well know that fruits initiated from early flowers have a lower probability of aborting than the fruits initiating late (Udovic and Aker 1981) which is mainly due to positional advantage (Bawa and Webb 1984, McNeilage 1991) and assured maternal investment.

Fruits in pink and green colours seems to quite unique in tamarind. Colour dimorphism in reproductive organs has been reported in temperate species (Steinhoff 1974, Farris and Mitton 1985) as a single gene inheritance (Steinhoff 1974). However this is probably the first report of fruit colour dimorphism being observed in a tropical tree.

CONCLUSIONS

Tamarind is a preferentially outcrossing species, it is self-incompatible with negligible amount of selfing. Low fruit set in open pollination seems to be a pollinator limitation. Controlled crossing yields nearly 90% fruit setting. Flowers being adapted with herkogamy and unique stamen arrangement are added advantages for outcrossing. As pollen storage is possible transfer of paternal germplasm is should be easier between locations. With a clearly understood breeding system and with standardised controlled pollinations techniques tamarind needs to be further exploited for its variations.

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SELECTION OF RAPD MARKERS FOR INVESTIGATION OF GENETIC POPULATION STRUCTURE IN FUSIFORM RUST FUNGUS INFECTING LOBLOLLY PINE

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Abstract.--Research to determine patterns of genetic differentiation among and within field populations of *Cronartium quercuum* f. sp. *fusiforme* using RAPD markers is currently underway in the molecular genetics laboratory at the Southern Institute of Forest Genetics. Fungal tissue was collected as a drop of spermatia or scrapings of a discrete hymenium from a single gall on each tree sampled at a location. Collections were made on twenty or more loblolly pines at 25 geographic locations, widely dispersed throughout the natural range of this host species. DNAs are presently being extracted from these tissue samples. The extracted DNAs are being amplified using the polymerase chain reaction (PCR) and 10-mer oligonucleotide primers to produce RAPD products that have potential for use as genetic markers. From bulked samples, twenty-one such RAPD markers have been identified that show consistent, clear band separations and polymorphisms that closely correspond to those produced by genetic markers previously shown to segregate as Mendelian factors in a *C. q. fusiforme* population derived from a single urediniospore culture. It is likely, however, that some of our extractions contain contaminant DNA, from host trees and also possibly from insects and other fungi. This extrinsic DNA might be amplified by PCR in addition to the targeted DNA. In this paper, we describe techniques that are being employed to provide reasonable assurance that the RAPD markers we use for analysis of allele and haplotype frequencies are *C. q. fusiforme* allelic segregants.

Keywords: *Cronartium quercuum* f. sp. *fusiforme*, genetic diversity, Mendelian segregation, polymerase chain reaction.

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INTRODUCTION

Pathogenic fungi are continually challenged to adapt to their environments, especially to that environmental component that is their host species. Those pathogens that attack forest tree species are no exception. Most are confronted by an array of environmental factors and infect host species that are genetically variable. Because they are constantly subjected to changes in their environments, these fungi must maintain the capability to evolve in order to survive. To have the ability to respond to such challenges, pathogen species must harbor a reservoir of genetic variation. Amounts of such variability as well as its distribution among and within populations are governed by evolutionary forces, working singly or in combination. These forces include genetic mutation, mating behavior, gene flow between populations, natural selection and population size effects. Ultimately, population geneticists that study pathogens of forest trees, hope to determine which of these factors are important in shaping genetic variability within most, if not all, of these fungal species.

An appropriate first step to determine how evolutionary forces affect pathogen populations is to study their genetic population structure (McDonald 1997). In the context of population genetics, population structure refers to the amount and distribution of genetic variability among and within populations and subpopulations. It is strongly influenced by the evolutionary history of the populations and provides information about their capacity to change in response to new evolutionary pressures. Information about the population structure of a pathogen is therefore needed to guide development of effective control measures for use in management of its pathosystem.

Fusiform rust disease caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f.sp. *fusiforme*, a heteroecious rust fungus, is a major threat to the health of loblolly pine (*Pinus taeda* L.) forests in southern United States. At present, the primary control measure consists of planting resistant trees developed through tree breeding. In these breeding efforts, however, control of the attacking pathogen's genetic constitution is not feasible and the genetic variability existing among and within the attacking populations is not known. As a consequence, little information is available regarding the nature of the resistances being deployed.

Currently, almost nothing is known about the genetic population structure for this pathogen. Several investigations, however, have uncovered evidence that suggests considerable genetic variability exists within this formae speciales. In studies of traits related to ability to cause disease, both gall development and percent infection were found to vary among collections originating from separate galls (Snow et al. 1975; Powers et al. 1977; Powers 1980; Kuhlman and Matthews 1993). Using RAPD markers in a preliminary study of population structure of *C. q. fusiforme* infecting loblolly pine, Hamelin et al. (1994) obtained results that indicate populations occurring east of the Mississippi river might be genetically differentiated from those coming from regions west of the river. Regional differention, however, accounted for only a small portion of the allele frequency variation observed; most of the genetic variation occurred within regions. These findings indicate that a more comprehensive study of population structure, based on neutral markers, is needed to clarify and further quantify patterns of genetic

differentiation that exist in *C. q. fusiforme* attacking loblolly pine. Such an investigation, using RAPD markers, is now underway at the Southern Institute of Forest Genetics.

SAMPLING METHODOLOGY

Fungal tissue was collected from galls on individual trees at 25 widely separated locations. Populations sampled were approximately 100 miles apart on a grid that spans most of the natural range of loblolly pine with major incidence of fusiform rust disease. For 23 of these populations, samples were taken from single galls on 20 trees. In each of the two other populations, Athens, GA and Saucier, MS, infections on more than 50 trees were sampled. In addition, multiple collections were made on some galls sampled in the Athens, GA population, so genetic variation within galls resulting from field infections may be detected if present.

Tissue was collected from each gall as a single drop of spermatia or as scrapings from a discrete hymenium. Spermatia in a single drop of fluid are believed to be produced by a single spermagonium and therefore to result from mitotic divisions originating from a single haploid mycelium (Mims and Doudrick 1996). Tissue obtained from a hymenial layer was taken from a restricted area (less than 10 mm^2) of a single hymenial zone occurring under the bark covering the surface of a gall. Each of these collections also is considered to be made up of cells derived from a single mycelium. Scrapings were not taken from a larger area because results obtained by Doudrick et al. (1993a) demonstrate that spermatial drops collected more than 10 mm apart can contain spores with different genetic constitutions, indicating that they are derived from mycelia produced by different infections.

Since we sampled tissue before the initial phase of sexual reproduction occurred, inferences drawn from our genetic analysis apply to the gametothallic haploid *C.q. fusiforme* populations that infect populations of loblloly pine. An advantage to concentrating effort on the gametothallic phase of the fungal life cycle is that multiple-locus haplotype frequencies can be studied in addition to allele frequencies. This makes it feasible to test for linkage disequilibrium, thereby gaining an additional analytical tool that may be used to help decipher genetic variation patterns.

RAPD MARKERS

Our individual tissue samples contain only small amounts of *C. q. fusiforme* biomass, consequently our genetic analysis must, by necessity, be based on molecular markers produced by polymerase chain reaction (PCR) methodology. RAPD markers resulting from PCR amplified DNA were used successfully by Doudrick et al. (1993a) to study genetic polymorphisms in a population of *C. q. fusiforme.* Methods developed in that research are readily available, making RAPDs the markers of choice for our investigation. Accordingly, using modifications of techniques described by those workers, DNA currently is being extracted from our samples and amplified using PCR employing 10-mer oligonucleotide primers to produce RAPD products. The resulting products are then separated by electrophoresis on

agarose gels and subsequently revealed by ethidium bromide staining and UV irradiation.

To perform analyses that will properly detect patterns of genetic variation that exist, we need marker polymorphisms that manifest consistent band profiles across a variety of genetic backgrounds and furthermore, that represent alleles which show Mendelian inheritance at individual segregating genetic loci. Such RAPD genetic polymorphisms are indicated by band presence or absence gels and are caused by nucleotide sequence variations or are products of DNA insertions or deletions between primer attachment sites (Clark and Lanigan 1993). An advantage that comes with analysis of haploid individuals is that we are not faced with the problem of dominance phenotypic expression that is troublesome when RAPD markers are used in genetic analysis of diploid eukaryotes or fungal heterokaryons.

MARKER ANALYSES

As a first step in the process to identify markers that satisfy the required criteria, we screened RAPD polymorphisms produced from bulked samples representing 24 of our populations. Each of these bulked lots contained DNA from three galls that are components of one of the population samples. Twenty one potential markers were identified in this screening process. These markers were polymorphic and showed clear, consistent bands that closely correspond to RAPD genetic markers that either have been included on a genetic map constructed for a segregating population of *C. q. fusiforme* (WLP-10-2.SS1) infecting loblolly pine (Doudrick et al. 1993b) or were found to be unlinked to the mapped loci. Both the mapped and unmapped markers were shown by those workers to demonstrate 1:1 band presence, band absence ratios in accordance with Mendelian segregation. Of our potential markers, six have bands that match those of loci mapped to four of the eight identified linkage groups, six match loci that mapped to linked pairs of loci and nine corresponded to loci that have not yet been assigned to a group or a linked pair.

To be reasonably sure that markers we use in our analysis are identical to genetic loci segregating in WLP-10-2.SS1 (WLP-10) and are not conflicted by spurious band polymorphisms, several check procedures are necessary in our DNA assays. A combined sample of WLP-10 DNA will be included in all of our assays to provide a standard for comparison purposes. Also all of the potential markers we have selected will be tested for authenticity by Southern hybridization analysis using DNA of the corresponding matching RAPD products from WLP-10 as probes. Those markers that fail to produce the expected hybridization pattern will be excluded from our genetical analysis. Moreover, there are two potential scoring problems that we must contend with; DNA contamination and PCR artifacts. Both can be eliminated as sources of error by Southern hybridization analysis.

Some of our extractions may contain contaminant DNA. In samples taken from hymenial tissue, pine host tissue might have been inadvertently collected along with fungal tissue. To check for this possibility, a bulked sample of loblolly pine DNA will be included in each of our RAPD assays. Markers found to have bands that migrate similar to amplified products produced by pine DNA sequences will be excluded from our analysis. In addition, samples obtained from

drops of spermatia may include DNA from insects or alien fungi. Such extrinsic DNA, however, is expected to be at low concentrations in our samples and to be only weakly amplified if at all because of competition for primers from the more highly concentrated target *C. q. fusiforme* DNA. This latter source of contamination is not expected to result in scoring problems. Samples that yield anomalous band profiles, that might be caused by either form of contamination will be discarded.

RAPD assays are sometimes subject to aberrant migration patterns that can lead to misclassification of phenotypes if caution is not used in scoring. This drawback to use of RAPD analysis is well known and can usually be traced to amplification irregularities or to nearly identical migration behavior of nonhomologous fragments. The review by Bachmann (1994) and discussion by Smith et al. (1994) describe the causes in detail and suggest procedures to be followed for elimination of mistakes. We are particularly concerned with two RAPD product detection problems that have surfaced in some of our assays. The first stems from competition for primers between annealing sites and is signaled by the appearance of one or more intensely bright bands. When such intense bands are observed but a RAPD product is not detected for the marker of interest, we can not determine whether the product is actually present but at such a low amplification that it can not be detected by ethidium bromide staining, or if it, in fact, is absent. The second problem involves comigrating nonhomologous products of similar but not equal molecular weight. Scoring mistakes arise when the product of interest is absent but its comigrating nonhomologue is present.

Both of the aforementioned detection problems can be resolved by preparing Southern blots using target RAPD products from WLP-10 as probes. The detection limit for Southern based systems (.01 ng) can be as high as 1000 times greater than that realized with ethidium staining. Furthermore, to insure that our genetic analyses are based upon data collected using rigorous methods, all samples yielding questionable assays will be subjected to Southern hybridization analysis using appropriate target DNA from WLP-10 as probes. Those that fail to yield expected hybridization patterns will be disregarded.

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CULTURE AND GENETIC VARIATION INFLUENCES ON JUVENILE WOOD PROPERTIES OF SLASH AND LOBLOLLY PINES IN FLORIDA

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Abstract. The effects of four cultural levels (conventional site preparation and bedding, maximal fertilization, complete vegetation control, and combined fertilization and vegetation control) on latewood percent, age of transition from juvenile to mature wood, and wood specific gravity of young slash and loblolly pines were first evaluated by extracting lower stem cores from 200 10-year-old trees on a flatwoods site near Gainesville, Florida. Latewood percent, which increased from ring ages 4 to 10 years, tended to increase with intensive culture, particularly in slash pine. Under the intensive cultures, slash pine formed mature wood starting at age seven, whereas loblolly pine did not typically form ≥50% latewood until nine years. No slash pine progeny formed mature wood before age seven, but two progenies tended to deposit more latewood at all ages. Findings on transition age generally confirm previous indications that slash and loblolly pines form mature wood earlier with decreasing latitude. Specific gravity in rings 6-10 was not related to tree DBH, total height, or height to live crown. A second sampling of 80 13-year-old trees using stem disks at 1.5m indicated a trend toward insignificantly lower specific gravity with intensive culture. Previous latewood and transition age patterns persisted. In both samples, slash pine had insignificantly higher specific gravity than loblolly pine in three cultures and overall. Variability between species and within slash pine for important wood properties may offset some undesirable wood quality changes resulting from culturally induced faster growth.

Keywords: *Pinus elliottii*, *Pinus taeda*, latewood, mature wood, specific gravity.

INTRODUCTION

Southern pine plantations are projected to provide 2.7 billion and 4 billion cubic feet, 50% and 67%, respectively, of harvested softwood in the Southeast in the years 2000 and 2030 (Knight 1986). This increasing reliance on plantation-grown trees with less desirable wood properties, such as the proportion of juvenile wood and the size and frequency of knots (Clark and Saucier 1991a), will have dramatic impacts on wood-using industries. As intensive culture and genetic improvement lead to more rapid tree growth in plantations, the consequences of this management intensity on the quality of an increasingly important wood resource become more important.

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This investigation had three objectives: 1) evaluate the effects of intensive culture, 2) assess the potential for genetic variability in slash pine to mediate culture-induced changes, and 3) extend previous indications of latitudinal influences for latewood percent (LWP), age of transition from juvenile to mature wood, and wood specific gravity of young slash and loblolly pines.

MATERIALS AND METHODS

The study, established in January 1983 at a spacing of 6' between trees along beds and approximately 12' between beds, is a split-plot design with three replications of the two species in main plots and four cultural treatments in subplots: conventional site preparation and bedding (C), maximal fertilization (F), complete vegetation control (H), and combined fertilization and vegetation control (F+H). Four progenies representing faster growing and more rust resistant clones were systematically located as 10 single-tree plots within slash pine subplots. The loblolly is a bulklot from an unrogued orchard of 30 clones (primarily Marion and Nassau Counties, Florida, selections), several of which were relatively rust-susceptible and slow-growing. The cultural treatments were maintained at least annually through 10 years.

For the first wood evaluation, two 12-mm bark-to-bark cores were taken in November 1992 at approximately 4.5' above ground from each of 200 10-year-old trees free of wood impacting stem diseases: 10 trees of each of four slash pine progenies and one loblolly bulklot per measurement subplot, for a total of 160 slash pine and 40 loblolly pine (Table 1a). Stratified random sampling was used to select two trees from the lower- through upper-1/5 of the DBH range in two replications of each culture of each of the five genetic entries. Cores were segmented into annual rings. Physical and anatomical measurements taken by the methods of Clark and Saucier (1989) were completed in June 1993. Each sample tree's DBH and some trees' total heights were measured after the wood sampling at age 10 years . Height to live crown, total height, and DBH at 11 and 13 years were also determined.

A second wood sampling in October 1995 used stem disks taken at 1.5m from 80 13-year-old trees (Table 1b) in gross plots surrounding the measurement subplots. The slash pine were taken without knowledge of specific pedigree from a mix of progenies 1-56 (33%), 6-56 (2%), 106-56 (32%), and 183-58 (33%), and the loblolly were the same orchard bulklot as in the measurement plots. Wood analyses again followed Clark and Saucier (1989). Latewood measurements utilized strips cut from disks.

RESULTS AND DISCUSSION

After 10 years, growth responses to cultural treatment were large, and species comparisons differed with treatment (Table 2). For slash pine, F or H increased volume/ha some two times over C. F+H resulted in as much as a four-fold increase in stand volume over C, depending on species. Slash pine progenies had approximately twice the volume/ha of the loblolly bulklot under C and were similar to loblolly with F or H. Slash progeny 6-56 had the greatest volume/ha under F+H. F+H produced trees of a size not typically achieved with conventional culture on flatwoods sites for at least another four years.

Table 1. Wood sampling conducted at a) 10 years of age for four slash pine progenies and a loblolly pine seed orchard bulklot and b) 13 years of age for slash pine and loblolly pine bulklots grown at four cultural levels..

Species: Progeny	Culture				Total
	C	F	H	F + H	
	(number of sample trees)				
a) Sampling at Age 10 from Measurement Plots					
Slash:					
1-56	10	10	10	10	40
6-56	10	10	10	10	40
106-56	10	10	10	10	40
183-58	10	10	10	10	40
Loblolly	10	10	10	10	40
Total	50	50	50	50	200
b) Sampling at Age 13 from Gross Plots					
Slash	10	10	10	10	40
Loblolly	10	10	10	10	40
Total	20	20	20	20	80

LWP generally increased with ring age, but the pattern of increase differed with species, progeny, and culture (Table 3). For slash pine, LWP averaged as low as 20% in C in the fourth year to as high as 59% in the ninth year with F. F, H, and/or F+H cultures significantly increased LWP at each age except five years, by as much as 10% at four years to just over 1% at 10 years. Differences among the intensive cultures varied with ring age but were usually small in slash pine. In loblolly pine, F, H, and F+H cultures had somewhat similar but inconsistent influences on LWP. The three intensive cultures usually resulted in nonsignificantly higher LWPs at all ages except age 5. H alone, though, tended to have more latewood in rings six through nine years. Increases in LWP in both species with more intensive culture may be due to F, H, and F+H cultures extending the growing season, as evidenced through additional growth flushes (Neary et al. 1990).

Intensive culture typically increased LWP at each age for each slash pine progeny (Table 3). However, the F, H, and F+H cultures had varying influence on the LWP of the four progenies. While progenies 106-56 and 183-58 tended toward higher amounts of latewood than progenies 1-56 and 6-56 at ring ages six through eight, progeny differences were nonsignificant. Progenies

Table 2. Tenth-year mean height, DBH, survival, basal area/ha, volume/ha, and rust and pitch canker incidences for slash pine progenies 1-56, 6-56, 106-56, and 183-58 and a loblolly pine seed orchard bulklot (S.O.Lob.) grown at four cultural levels.

Genetic Entry	Height (m)	DBH (cm)	Survival (%)	BA/ha (sq.m)	Vol/ha (cu.m)	Rust (%)	Pitch Canker (%)
				C			
1-56	8.9	10.2	90.0	11.7	46.1	25.0	0.0
6-56	10.3	12.4	96.7	17.7	103.3	23.3	0.0
106-56	10.2	10.8	83.3	12.0	83.0	18.5	0.0
183-58	9.4	11.1	83.3	12.3	76.3	24.0	4.0
S.O.Lob.	8.1	9.0	92.5	9.8	43.8	40.5	0.0
				F			
1-56	12.1	14.7	86.7	22.8	111.2	42.9	0.0
6-56	13.6	15.0	93.3	25.2	201.2	17.9	0.0
106-56	12.3	15.6	83.3	24.3	151.1	15.4	0.0
183-58	11.1	15.0	76.7	21.0	127.9	41.7	8.3
S.O.Lob.	13.7	15.7	92.5	28.2	168.5	61.9	0.9
				H			
1-56	10.9	14.2	83.3	20.4	123.5	23.1	3.8
6-56	13.0	15.0	83.3	22.8	151.7	29.6	0.0
106-56	12.7	14.7	96.7	25.2	166.5	40.0	3.3
183-58	12.9	15.0	90.0	26.4	186.7	37.9	0.0
S.O.Lob.	12.7	15.7	92.5	24.6	158.4	65.2	0.0
				F + H			
1-56	13.3	16.4	70.0	22.4	131.5	28.0	16.0
6-56	13.3	18.8	96.7	40.4	270.7	23.3	20.0
106-56	11.1	16.7	70.0	23.8	153.4	35.7	22.2
183-58	11.9	17.7	90.0	33.5	183.0	41.4	31.0
S.O.Lob.	14.4	17.0	93.3	32.4	195.6	53.5	0.9

106-56 and 183-58 formed as much as 63% latewood in the ninth annual ring when grown in F or H culture. Differences among progenies in LWP are moderately heritable, but latewood density is more heritable than LWP (Hodge and Purnell 1993).

Cregg et al. (1988) noted that low rainfall and/or high evaporation may delay a tree's transition from earlywood to latewood in a particular year by up to a month. The differences from year to year in LWP, particularly from ring 9 to 10 (Table 3), may reflect a dry summer in 1992. Cregg speculated that summer rains may extend latewood formation in the Coastal Plain.

Using 50% latewood as the transition from juvenile wood to mature wood (McAlister and Powers 1992), intensive culture influences the length of the juvenility period in both species (Table 3). Under conventional culture, slash pine did not average 50% latewood at any age

Table 3. Fourth- to 10th-year latewood percentages for four slash pine progenies, slash pine overall, and a loblolly pine seed orchard bulklot grown at four cultural levels.

Genetic Entry	Latewood Percentage by Annual Ring Age (years) 4	5	6	7	8	9	10
	----------C----------						
1-56	15.7	22.0	28.3	41.6	43.0	51.5	49.1
6-56	20.9	22.3	27.7	42.3	34.6	46.8	41.9
106-56	17.1	25.1	36.0	47.6	45.9	49.8	51.6
183-58	25.2	26.8	34.3	47.8	46.2	50.7	46.9
Slash	19.9b[1]	24.0a	31.6b	44.8b	42.4b	49.7c	47.4c
Loblolly	13.4a	18.0a	21.8b	34.6a	42.5a	44.0a	38.2a
	----------F----------						
1-56	28.1	28.2	39.8	56.3	49.0	58.4	47.6
6-56	34.0	28.4	30.1	47.9	46.0	55.1	46.3
106-56	31.4	28.5	40.6	56.6	58.1	63.2	54.9
183-58	27.9	27.8	39.5	51.6	55.7	60.5	47.0
Slash	30.4a	28.2a	37.5ab	53.1a	52.2a	59.3a	48.9bc
Loblolly	15.4a	18.4a	27.9ab	38.8a	42.8a	53.2a	50.2a
	----------H----------						
1-56	26.4	24.5	42.3	49.0	45.7	57.9	49.0
6-56	27.8	22.8	34.4	49.0	47.6	52.8	41.2
106-56	32.0	26.3	41.4	59.3	52.6	62.0	51.0
183-58	34.5	33.8	44.9	54.9	55.0	62.6	55.1
Slash	30.2a	26.9a	40.8a	53.1a	50.2a	58.8a	49.1ab
Loblolly	18.7a	18.1a	34.7a	50.7a	51.4a	57.7a	49.1a
	----------F + H----------						
1-56	24.2	22.8	37.1	52.4	48.1	54.2	47.1
6-56	28.3	27.4	36.2	56.5	45.4	55.7	51.4
106-56	25.3	24.4	39.8	55.9	52.1	60.2	50.7
183-58	34.6	29.4	41.9	57.8	55.0	61.3	53.4
Slash	28.2a	26.0a	38.7ab	55.6a	50.2a	57.9b	50.6a
Loblolly	25.7a	14.8a	26.6ab	42.8a	44.9a	52.9a	47.9a
	----------Overall----------						
Slash	27.2a;E[2]	26.3a;E	37.2a;D	51.7a;B	48.7a;C	56.4a;A	49.0a;C
Loblolly	18.9a;D	17.3b;D	27.8a;C	41.7b;B	45.4a;AB	52.0a;A	46.4a;AB

[1]Within a species and age, culture means not sharing a lower case letter are significantly different at the 5% level by Duncan's Multiple Range Test

[2]Within an age, species means not sharing a lower case letter are significantly different at the 5% level by Duncan's Multiple Range Test; Within a species, age means not sharing an upper case letter are significantly different at the 5% level by Duncan's Multiple Range Test

except nine years. However, F, H, and F+H each formed mature wood at age seven years. This agrees with the Clark and Saucier (1991a) expectation that slash pine produces juvenile wood for six years in Florida. Intensive culture did not cause any of the slash pine progenies to exceed 50% latewood before seven years, but progenies 106-56 and 183-58 tended to have high LWPages, even over 60%, beginning in the seventh year when grown intensively. Mature wood classified by Clark et al. (1990) had approximately 53% latewood. Intensive site preparation increased the duration of juvenility of slash pine by two years on moderately drained Coastal Plain sites (Clark et al. 1990).

The juvenile period for loblolly pine in Florida is at least six years, which is the age reported by Clark and Saucier (1991a) for slash in Florida. With complete vegetation control, loblolly began to form mature wood in the seventh year. With complete fertilization, the transition to mature wood may have begun in the ninth year. Thus, unlike the observation of Clark and Saucier (1991a), intensive culture may influence the duration of juvenility in loblolly pine. Clark and Saucier (1991b) suggested that the duration of juvenility in loblolly pine may be related to the length of the growing season and seasonal rainfall pattern. These findings on transition age generally confirm previous indications that slash and loblolly pines form mature wood earlier with decreasing latitude.

Through 14 years, the F, H, and F + H treatments continued to have significant effects (Anon., 1997). The F and H treatments gave similar stand volume increases over C in both slash and loblolly pine, although the F treatment had insignificantly higher volume in loblolly. Compared to C, the F + H treatment increased volume 2.2-fold in slash pine to 2.9 cords/acre/year, while in loblolly pine, stand volume increased 3.6-fold to 3.3 cords/acre/year. As a percentage of total aboveground biomass, slash pine averaged 17.2% bark across treatments in contrast to 12.1% for loblolly. Stemwood growth efficiency was higher for loblolly pine.

Allowing for the potentially different progeny representation for slash pine in the age 13 years wood sample, trends in LWP were generally consistent with the earlier sampling (Table 4). Through 10 years, slash pine had higher LWP than loblolly at virtually every culture and age combination, especially at young ages. After 10 years, LWP tended to increase, and loblolly typically had insignificantly higher LWP than slash. At age 13, culture had no influence on LWP in slash, but more intensive culture resulted in higher LWP in loblolly.

The LWP responses in the two samples were somewhat reflected in the wood specific gravities determined (Table 5). For annual rings 6 to 10, LWP and specific gravity were positively associated. For most cultures and/or species, LWP was a good indicator of wood density. Under the H culture, specific gravities were generally higher and less reflective of LWP.

Based on the first 13 annual rings combined, LWP was less useful in predicting specific gravity. Perhaps due to inconsistent progeny representation across cultures, LWP and specific gravity differences were largely insignificant. For loblolly, the H culture tended toward a higher density.

Table 4. Fourth- to 13th-year latewood percentages for slash pine and loblolly pine bulklots grown at four cultural levels.

Genetic Entry	Annual Ring Age (years) 4	5	6	7	8	9	10	11	12	13
	----------C----------									
Slash	10.5b[1]	14.9b	22.4a	41.2a	36.4a	42.6b	46.5a	40.0a	51.3a	47.6a
Loblolly	12.9a	9.3a	15.4b	29.8b	38.6a	41.9a	42.0a	39.9a	59.4a	52.5b
	----------F----------									
Slash	25.3a	34.7a	39.3a	46.4a	50.4a	59.3a	51.4a	45.4a	58.3a	54.1a
Loblolly	13.3a	14.5a	25.6a	39.1a	38.8a	46.3a	47.1a	45.9a	56.0a	55.0ab
	----------H----------									
Slash	17.8ab	19.5b	31.9a	43.6a	47.3a	56.6a	49.3a	40.5a	53.7a	49.8a
Loblolly	15.3a	13.9a	23.6a	38.5a	43.4a	51.2a	45.1a	44.8a	57.2a	60.4a
	----------F + H----------									
Slash.	17.2ab	23.0ab	35.1a	46.5a	48.4a	58.1a	49.0a	43.2a	55.2a	45.9a
Loblolly	15.1a	13.7a	25.5a	37.7a	44.9a	53.8a	47.9a	45.8a	60.3a	56.4ab
	----------Overall----------									
Slash	17.9aCD[2]	23.0aCD	32.2aBC	44.4aAB	45.7aAB	54.1aA	49.0aA	42.9aAB	54.6aA	49.3aA
Loblolly	14.2aEF	12.8aF	22.5bE	36.3bD	41.4aCD	48.3aBC	45.5aCD	44.1aCD	58.2aA	56.1aAB

[1]Within a species and age, culture means not sharing a lower case letter are significantly different at the 5% level by Duncan's Multiple Range Test

[2]Within an age, species means not sharing a lower case letter are significantly different at the 5% level by Duncan's Multiple Range Test; Within a species, age means not sharing an upper case letter are significantly different at the 5% level by Duncan's Multiple Range Test

Specific gravity in the lower stem did not appear to be associated with tree size or crown height. The specific gravity of rings 6-10 was not significantly correlated with tree DBH, total height, or height to live crown (Table 5).

The intensive cultures previously reported not to have significantly changed specific gravity (Clark and Saucier 1991a) were not as intensive as the treatments applied in this study. F here was a complete fertilizer formulation applied several times a year for eight years beginning at establishment. Similarly, H was complete vegetation control from the beginning using herbicides and mechanical means.

The increased volume and weight yields (wood + bark) from intensive culture may be supplemented by favorable changes in bark volume and weight. In contrast to this study, for 11-year-old loblolly pine in Louisiana of virtually the same size as the F+H cultured loblolly here, Tiarks and Haywood (1993) found that fertilizer and competition control increased wood weight per stem volume by 2.8% and decreased bark volume by 15%.

Table 5. Wood specific gravity and ring diameter and basal area for ring ages six to 10 years and height to live crown at age 11 years for slash pine progenies 1-56, 6-56, 106-56, and 183-58 and a loblolly pine seed orchard bulklot and wood specific gravity, moisture content, and ring basal area for rings one to 13 years for slash and loblolly pine bulklots grown at four cultural levels.

	Ring Ages 6 to 10 Years			Height	Ring Ages 1 to 13 Years		
Genetic Entry	Specific Gravity (g/cm^3)	Ring Diameter (cm)	Ring Basal Area (sq. cm)	to Live Crown (m)	Specific Gravity (g/cm^3)	Moisture Content (%)	Ring Basal Area (sq. cm)
				C			
1-56	.534	5.14	8.99	4.84			
6-56	.497	6.34	14.18	5.25			
106-56	.553	5.96	11.81	4.98			
183-58	.545	5.42	10.43	5.28			
Slash	.533[1]			5.08	.541a	90.7a	.111c
Loblolly	.503	6.10	8.46	2.45	.538a	89.1a	.069d
				F			
1-56	.509	5.50	14.97	6.51			
6-56	.508	6.10	18.35	7.18			
106-56	.562	5.50	16.14	6.77			
183-58	.536	6.09	18.39	6.93			
Slash	.529			6.85	.546a	86.9a	.159b
Loblolly	.512	6.86	20.99	7.64	.518a	95.3a	.155c
				H			
1-56	.544	5.07	14.59	6.78			
6-56	.525	5.48	16.43	7.46			
106-56	.574	5.36	15.79	7.04			
183-58	.580	5.63	17.37	6.99			
Slash	.556			7.07	.532a	92.2a	.160b
Loblolly	.557	5.64	15.92	5.30	.540a	90.2a	.191b
				F + H			
1-56	.518	5.13	17.14	7.24			
6-56	.515	6.31	24.40	7.50			
106-56	.541	4.95	17.21	7.11			
183-58	.542	5.85	20.62	7.24			
Slash	.529			7.27	.514a	92.9a	.221a
Loblolly	.508	5.45	17.53	7.72	.503a	102.7a	.256a
				Overall			
Slash	.536A[2]			6.59A	.533A	90.7B	.168A
Loblolly	.520A			5.45A	.525A	94.3A	.163A

[1]Culture means within a species for a trait not sharing a lower case letter are significantly different at the 5% level by Duncan's Multiple Range Test

[2]Species means within a trait not sharing an upper case letter are significantly different at the 5% level by Duncan's Multiple Range Test

Genetic variation may provide opportunities for combining desirable wood properties with fast growth and disease resistance. In five- and six-year-old loblolly pine, provenances and families differed in date of height growth cessation and date of latewood transition, and significant correlations were evident for later height growth cessation, later latewood transition, and lower wood specific gravity (Jayawickrama et al. 1995). In a wide sampling of loblolly families and sites, large family differences in wood density were observed, stem volume was slightly negatively correlated with wood density, and sites had a major impact on density (Belonger et al. 1996). Transition age to mature wood is heritable in loblolly pine (Loo et al. 1985). Slash pine progenies such as 106-56 and 183-58 that continue to form dense wood under intensive culture hold such potential, whereas progeny 6-56 demonstrates a very responsive genotype that does not mediate culture-induced change in wood density.

Clark and Saucier (1991a) identified that initial planting density, thinning regime, and rotation length influence wood properties and that length of growing season (latitude or geographic location) affects duration of juvenility. They suggest that plantations need to be managed for No. 2 or better lumber.

Lumber yield is influenced by the formation of clear mature wood (Clark and Saucier 1991b). Initial planting density (to control the diameter of the juvenile wood core and branch diameter and to encourage pruning), thinning regimen (to promote the production of clear wood along the lower stem), and rotation length (to increase the volume of mature wood along the stem) are silvicultural tools to influence the yield of high quality lumber in plantations. When applied to the intensively cultured slash and loblolly pines planted at 1,500 trees/ha in this study, these guidelines would call for a thinning at a relatively young age, perhaps seven years in slash pine and nine years in loblolly pine. Wood accumulation after these ages would be largely mature wood on stems with few branches.

CONCLUSIONS

Latewood percent, which increased from ages 4 to 13 years, tended to increase with intensive culture, particularly in slash pine. Under the intensive cultures, slash pine formed mature wood starting at age seven, whereas intensive culture of loblolly pine did not typically result in ≥50% latewood until nine years. No slash pine progeny formed mature wood before age seven, but two progenies tended to deposit more latewood at all ages. Variation among slash pine progenies may provide opportunities for combining desirable wood properties with fast growth and rust resistance. Progenies such as 106-56 and 183-58 that continue to form dense wood under intensive culture hold such potential, whereas progeny 6-56 demonstrates a very responsive genotype that does not mediate culture-induced change in wood density.

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FUSIFORM RUST EPIDEMICS IN FAMILY MIXTURES OF SUSCEPTIBLE AND RESISTANT SLASH AND LOBLOLLY PINES

R. A. Schmidt[1] and J. E. Allen[2]

Abstract. Fusiform rust incidence (% trees infected) was measured through age 5 yr for mixtures of susceptible and resistant slash pine (*Pinus elliottii* var. *elliottii*) and loblolly pine (*P. taeda*) planted in seven potentially high-rust-hazard locations in the Coastal Plain of FL, GA and MS. Four mixtures of six open-pollinated pine families were evaluated: rust-susceptible slash (SS), rust-resistant slash (RS), rust-susceptible loblolly (SL), and rust-resistant loblolly (RL). Few trees were infected through age 2 yr; rust increased in years three to five concomitant with an increase in tree height. Average rust incidence at age 5 yr was 46.5, 16.0, 44.2 and 10.4% for SS, RS, SL and RL, respectively. Resistant mixtures had significantly less rust than susceptible mixtures at all locations. Few interactions between rust incidence and age occurred among mixtures within locations. At three locations, slash pine was significantly more infected than loblolly pine; at one location, loblolly pine was significantly more infected than slash pine. Rust incidence on susceptible family mixtures varied among locations reflecting the rust hazard of the site. Rust incidence on RS mixtures varied among locations, increasing significantly with increasing rust hazard, i.e., with increasing rust incidence on SS family mixtures. Rust incidence on RL mixtures increased slightly with increased rust incidence on SL, but differences among locations were not significant. These results suggest that similar mixtures of half-sib rust-resistant slash and loblolly pine families will reduce rust incidence substantially in a variety of locations and rust hazards, but the performance of resistant slash pine family mixtures may be reduced significantly in locations with very high rust incidence.

Keywords: *Cronartium quercuum* f. sp. *fusiforme, Pinus elliottii* var. *elliottii, Pinus taeda*, stability of rust resistance.

INTRODUCTION

Fusiform rust of slash pine (*Pinus elliottii* Engelm. var. *elliottii)* and loblolly pine *(P. taeda* L.) caused by the obligate parasite *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme,* results in millions of dollars of losses annually in the southeastern USA (Anderson *et al.* 1986, Powers *et al.* 1975). Fusiform rust, of rare occurrence at the beginning of this century (Griggs and Schmidt 1977), apparently spread from west to east (Mississippi to Atlantic Coast) (Dinus 1974) and increased dramatically as pine management intensified (Schmidt 1978). The fungus exhibits variability in pathogenicity (Kuhlman 1990, Powers 1980) and half-sib (open-pollinated) pine families respond differentially to fungus isolates (Powers 1985). Genetic resistance to the pathogen occurs in slash and loblolly pine (Schmidt and Goddard 1971, Wells and Wakely 1966) and resistant pines are planted to reduce rust impact (Schmidt *et al.* 1985).

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Notwithstanding successes in finding and using fusiform rust resistance, information is needed on the temporal and spatial stability of widely-planted, intensively-managed resistant pine genotypes. The factors affecting stability in the field are especially important now that a major gene for resistance has been identified in the fusiform rust - loblolly pine pathosystem (Wilcox *et al.* 1995), and previously in the white pine blister rust - sugar pine pathosystem (Kinloch and Comstock 1981, Kinloch *et al.* 1970).

Our objective was to assess the disease progress and spatial stability of rust resistant slash and loblolly pine mixtures planted at seven locations in the Southeastern Coastal Plain exhibiting different levels of rust incidence, i.e., rust hazard, and cultural treatments.

METHODS AND MATERIALS

The general methods and materials were reported in previous publications on the influence of oak management on fusiform rust incidence (Schmidt *et al.* 1993) and the stability of resistance in slash and loblolly pine (Hodge *et al.* 1993). Only those methods appropriate to the data on epidemics in resistant and susceptible pine family mixtures are presented here.

Family mixtures. Pine seedlings of four mixtures - rust-susceptible slash (SS), rust-resistant slash (RS), rust-susceptible loblolly (SL) and rust-resistant loblolly (RL) - were planted in six locations in FL and GA (Figure 1). At the seventh location in MS only loblolly mixtures were planted. Original selections were from the Coastal Plain of NC, SC, FL, GA, AL and MS (see source, Figure 1). Seed were collected from open-pollinated clones established in seed orchards. Seedlings were grown in a nursery in southcentral GA in the spring and planted at the test locations the following winter. One location was planted in 1985, four were planted in 1986 and two were planted in 1987 (Table 2).

Each of the four mixtures consisted of a random mix of equal proportions of seed from six very susceptible or very resistant open-pollinated families, as determined in field progeny trials and/or greenhouse tests. The average rust indices (Hatcher *et al.* 1981) were 92, 28, 77 and 32 for RS, SS, RL and SL family mixtures, respectively (Table 1).

Table 1. Average fusiform rust index for family mixtures of susceptible or resistant slash and loblolly pines planted in seven high-rust-hazard locations in the Coastal Plain of FL, GA and MS.

Family mixture	No. of families in mixture	Rust index[1]	
		Ave.	Range
Resistant slash (RS)	6	92	87-99
Susceptible slash (SS)	6	28	15-39
Resistant loblolly (RL)	6	77	73-86
Susceptible loblolly (SL)	6	32	19-41

[1]NC State Tree Improvement Cooperative rust index values for loblolly pine (Hatcher *et al.* 1981) and those calculated for slash pine from the University of FL Cooperative Forest Genetics Research Program. An average family (unimproved for rust resistance) would have a value of 50: higher values = greater resistance.

Experimental design and analyses. At each location, two 3.64 ha (9.0 ac) areas, each consisting of thirty-six 0.10 ha (0.25 ac) subplots were installed (Figure 1). One area was treated annually to remove susceptible oaks; in the other area, oaks were allowed to grow. Each area accommodated a 2 (species) x 2 (family mixtures) x 3 (cultural treatments) randomized complete block split-split plot design with three replications. Each area consisted of six main plots arranged in three replications. The main plot was pine species, split into resistant and susceptible pine mixtures. Three cultural treatments were applied at planting to these split plots: control (no treatment); fertilizer + herbicide, and fertilizer + herbicide + Bayleton®. There were 36 subplots on each area or a total of 72 subplots at each location. A preliminary analysis indicated that there were few consistent relations in percentage trees infected or height among the cultural treatments and unless otherwise noted, these treatments were pooled to analyze family mixtures. Thus, there were 72/4 or 18 subplots/family mixture. Data were collected from a 54-tree sample at the center of each subplot of 164 trees. The number of branch and stem galls on each tree was recorded annually at ages 1-5 yr and cumulative rust incidence (% trees infected) was calculated. Tree height was measured at ages 1, 3 and 5 yr.

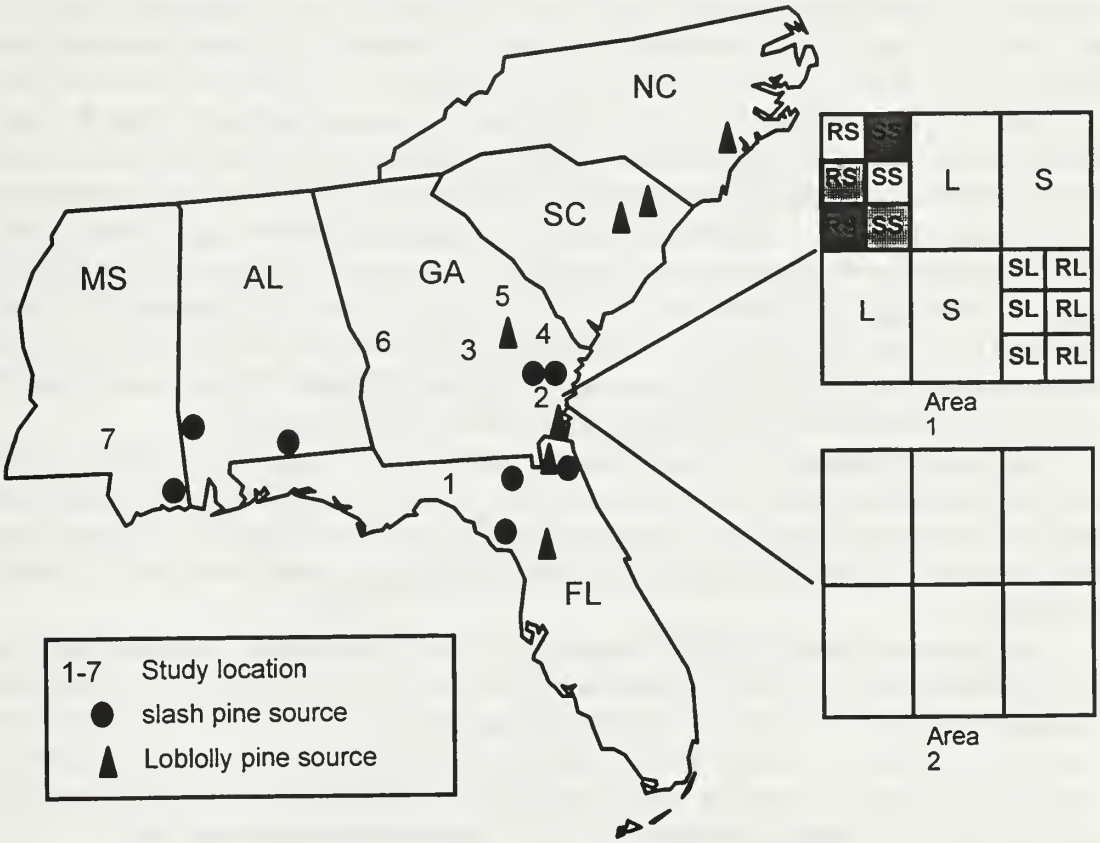

Figure 1. Fusiform rust study locations (1-7) and geographic sources of pine selections in the Southeastern Coastal Plain and experimental design. Increasing intensity of shading represents increasing intensity of cultural treatments (described in text). All replications were similar to the one shown shaded with the exception that Area 1 was treated annually to eliminate oak while Area 2 was not treated for oak control. SS = susceptible slash, RS = resistant slash, SL = susceptible loblolly and RL = resistant loblolly.

Differences in percentage trees infected within and among locations were examined with analysis of variance (Anonymous 1985). Differences in percentage trees infected between species at each location were determined by the F-test using the appropriate mean square error term. Significant differences in percentage trees infected among locations for each family mixture were determined conservatively, using Duncan's Multiple Range test with the location x area interaction mean square as the error term. Because rust incidence on susceptible mixtures (rust hazard) varied among locations, the relative resistance, i.e., percentage of trees infected in resistant mixture/percentage of trees infected in susceptible mixture (R/S ratio) was used in an analysis of variance and regression analyses to evaluate the stability of rust resistance among locations and treatments.

RESULTS

Disease progress age 1-5 Yr. Rust incidence on susceptible mixtures (SS, SL), indicative of rust hazard, remained low through 2 yr at five locations; at Perry and Screven, rust incidence was moderate on these mixtures by age 2 yr (Figure 2). Rust increased substantially on SS and SL mixtures at all locations subsequent to age 3 yr. The pattern of rust increase on these susceptible mixtures after age 2 yr varied among locations; at Perry and Louisville there was little or no increase in rust from yr 3 to 4; at Statesboro, a substantial increase in rust occurred from yr 3 - 5; at McRae, Perkinston and Americus, substantial rust occurred during yr 4 and 5. Rust on SS and SL mixtures increased substantially during yr 4 only at Screven and yr 5 only at Louisville. Overall infection during the first 3 yr resulted in 70 and 43% of the branch galls growing into the stem by age 4 yr at Perry, FL and McRae, GA, respectively, while by age 4 yr only 20% of the branch galls had grown into the stem at Statesboro. Among all locations, the average ranking at age 5 yr, from high to low rust incidence was: SS, SL, RS and RL, although SL $\geq$ SS at two locations (Screven and Louisville). Family mixtures maintained their relative positions of rust incidence throughout age 5 yr with the exception of SL during the fifth year, when at six of seven locations, SL exhibited a differentially high increase in rust incidence.

Comparison of species. Average cumulative rust incidence at age 5 yr on slash pine (SS, RS) was 30.9% and was significantly greater than that (26.4%) on loblolly pine (SL, RL) among all locations, and within locations at Statesboro, McRae and Perry (Table 2). Average cumulative rust incidence on loblolly pine (SL, RL) was significantly greater than that on slash pine at Louisville.

Comparisons of family mixtures. In both species and at all locations, resistant family mixtures had significantly less rust than susceptible family mixtures (Figure 2, Table 2). Among locations, SS ranged from 26.3 - 76.1%, $\bar{x}$ = 46.5%; RS ranged from 5.3 - 34.3% $\bar{x}$ = 16.0%; SL ranged from 22.8 - 62.7%, $\bar{x}$ = 44.2%, and RL ranged from 5.3 - 14.4%, $\bar{x}$ = 10.4% (Table 2). Rust incidence on SS and SL mixtures varied significantly among locations (reflecting rust hazard of the locations). Correlation between SS and SL for rust incidence was 0.86, $p = 0.03$. Rust incidence on the RS mixture varied significantly among locations and was correlated ($r = 0.97$, $p = 0.001$) with rust incidence on the SS mixture. Rust incidence on the RL mixture varied little among locations, but was correlated ($r = 0.92$, $p = 0.003$) with rust incidence on the SL mixtures.

Relative resistance of family mixtures (resistance/susceptible ratio). Relative rust resistance (RS/SS ratio of cumulative percentage trees infected at age 5 yr) of slash pine ranged from 0.20 - 0.45 among locations, varied significantly among locations (Table 2) and was correlated ($r = 0.80$,

p - 0.06) with rust incidence on SS. In loblolly pine, the RL/SL ratio ranged from 0.18 - 0.27 among locations, did not vary significantly among locations (Table 2) and was not correlated (r = 0.10, p = 0.83) with rust incidence on SL. The average ratios for slash pine (0.34) and for loblolly pine (0.23) were significantly different (p - 0.05).

Rust incidence in relation to pine growth (height), age and cultural treatments. The positive relation between rust incidence (total number of galls) and height at age 1, 3 and 5 yr at Statesboro, GA for slash pine (Figure 3A) and loblolly pine (Figure 3B) was typical of all locations in that it reflects the increase in rust susceptible tissue with increasing age. The associated number of stem galls at age 5 yr at Statesboro for SS, RS, SL and RL was 253, 126, 152 and 31, respectively. The number of total galls (branch and stem) per infected trees at age 5 yr for SS, RS, SL and RL was 3.6, 2.4, 2.8 and 1.6, respectively. Within species, there was no significant difference between the average heights of susceptible and resistant family mixtures at ages 1, 3 or 5 yr for slash or loblolly pine. Loblolly pine was significantly taller than slash pine at age 5 yr at Statesboro, but this relation between species varied among locations.

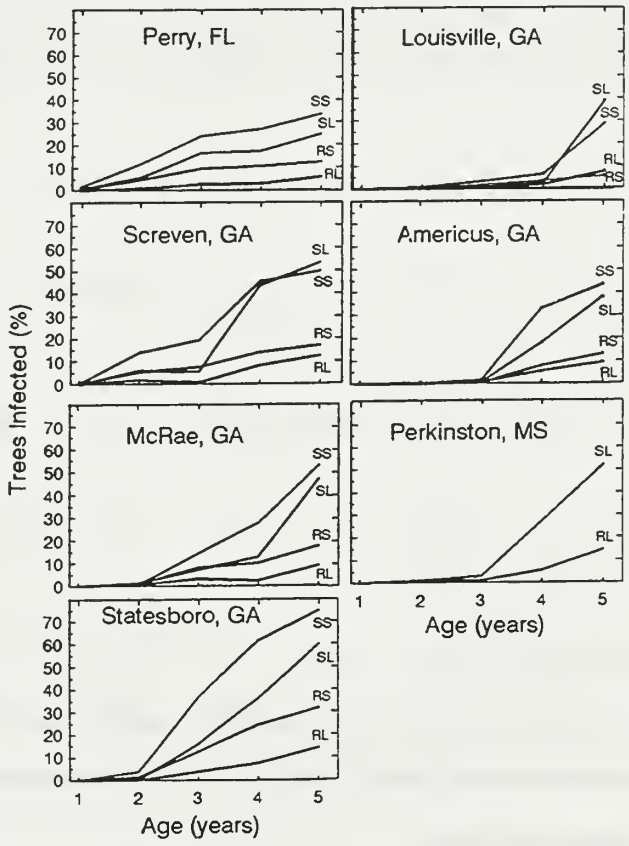

Figure 2. Disease progress (cumulative percentage trees infected) from ages 1-5 yr in family mixtures of rust-resistant or susceptible slash and loblolly pines planted (1985-87) at seven locations in the Coastal Plain of FL, GA and MS. SS = susceptible slash, RS = resistant slash, SL = susceptible loblolly, and RL = resistant loblolly.

Table 2. Fusiform rust incidence (cumulative percentage trees infected) and relative performance (resistant/susceptible ratio) at age five years in mixtures of open-pollinated progenies of resistant and susceptible slash and loblolly pines planted at seven locations in the Coastal Plain of FL, GA and MS[1].

	Year	Average		Slash		Loblolly		Resist/Susc ratio	
Locations[2]	planted	Slash	Loblolly	Susc.	Resist.	Susc.	Resist.	Slash	Loblolly
Statesboro, GA									
Bulloch Co. (4)	86	59.5A[3]	38.4B	76.1a[4]	34.3a	62.7a	14.1a	0.45a	0.22a
McRae, GA									
Wheeler Co. (3)	86	35.8A	26.4B	53.6b	18.0b	44.7bc	8.6a	0.34ab	0.19a
Screven, GA									
Wayne Co. (2)	86	32.1A	32.4A	49.1b	15.1bc	52.0abc	12.8a	0.31ab	0.25a
Perkinston, MS									
Stone Co. (7)	87					54.5ab	14.4a		0.26a
Americus, GA									
Sumter Co. (6)	87	26.6A	24.6A	42.4bc	11.6bc	36.0cd	9.7a	0.27ab	0.27a
Perry, FL									
Taylor Co. (1)	85	21.6A	14.0B	31.7cd	11.4bc	22.8d	5.3a	0.36ab	0.23a
Louisville, GA									
Jefferson Co. (5)	86	16.2B	24.0A	26.3d	5.3c	37.2cd	7.0a	0.20b	0.18a
Average		30.9A	26.4B	46.5A	16.0B	44.2A	10.4B	0.34A	0.23B

[1]Average of 18 plots (excluding missing plots) per family mixture: 54 measurement trees per plot (excluding rust-unrelated mortality).

[2](#) refers to study location in Figure 1.

[3]Means within each location (within each row) followed by different capital letters are significantly different (p = 0.05) according to F-test.

[4]Means among locations (within a column) followed by different lower-case letters are significantly different (p = 0.05) according to Duncan's Multiple Range test.

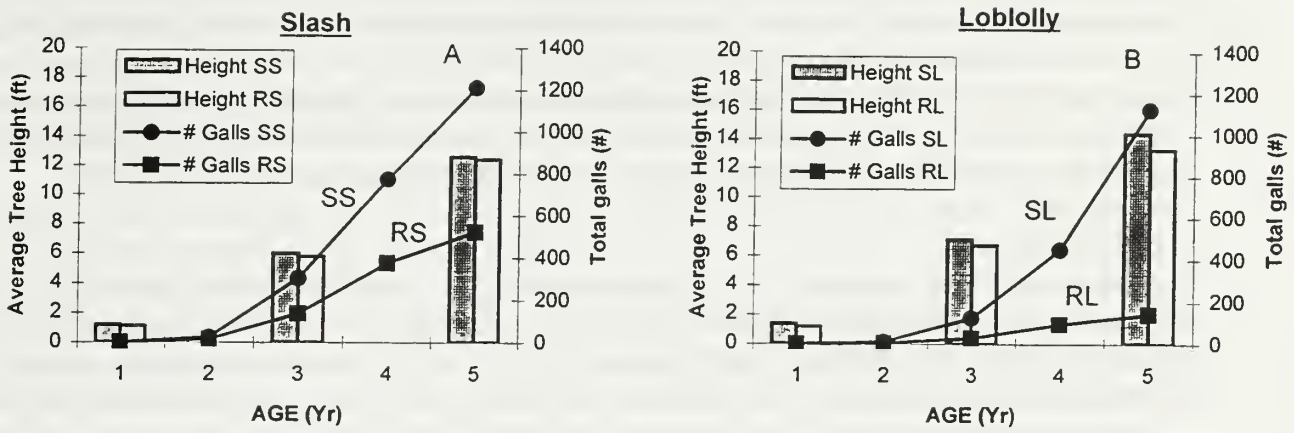

Figure 3. Cumulative fusiform rust incidence (total number of galls {branch and stem}) at ages 1-5 yr and height at ages 1, 3 and 5 yr of rust-susceptible and rust-resistant slash pine (A) and loblolly pine (B) family mixtures at Statesboro, GA.

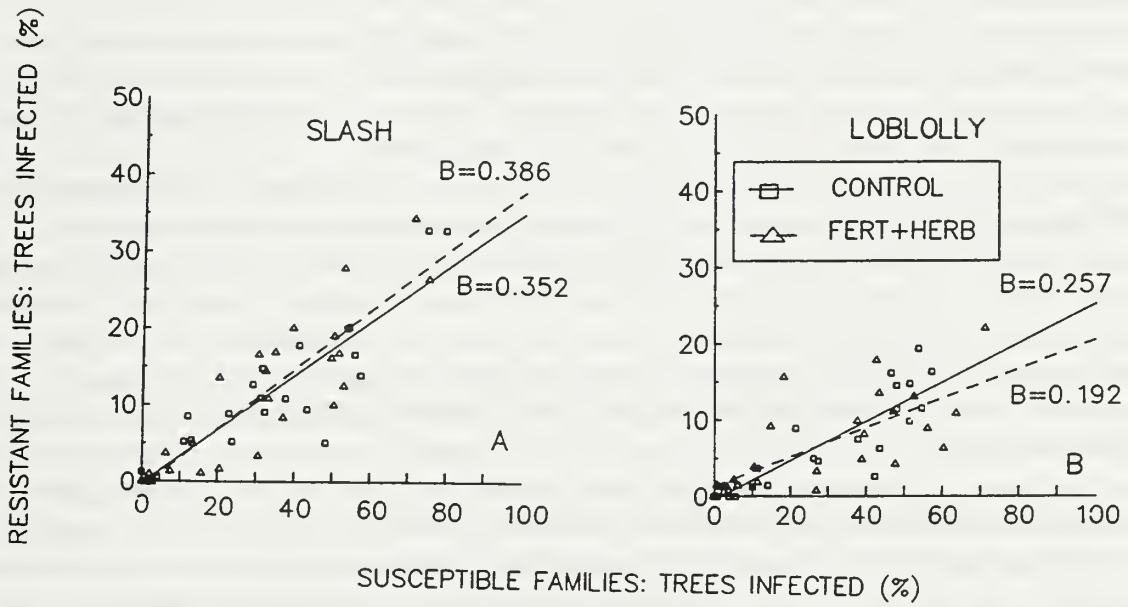

Figure 4. Relationship between cumulative percentage trees infected at age 3 and 5 yr on fusiform rust susceptible and resistant slash pine family mixtures (A) and susceptible and resistant loblolly pine family mixtures (B) receiving cultural treatments to promote growth at high-rust-hazard locations in the Coastal Plain of FL, GA and MS. Control = no treatment; Fert + Herb = fertilizer and herbicides applied at planting.

The relation among cultural treatments (fertilizer and herbicides vs control) at planting, pine height and rust incidence varied among locations. At some locations, fertilizer and herbicide increased pine heights significantly over that of the control with or without a significant increase in rust incidence. At other locations, fertilizer and herbicide did not increase pine height significantly with or without significant increases in rust incidence. Overall, this variation resulted in a linear R/S ratio with increased rust incidence, and did not result in significant differences in regression coeficients for slash pine (Figure 4A) or loblolly pine (Figure 4B).

DISCUSSION

Rust incidence was low at all locations through age 2 yr and increased substantially during ages 3-5 yr at most locations. Others (Griggs *et al.* 1978, Schmidt *et al.* 1979) reported similar patterns of disease progress for fusiform rust. Our pine height data suggest that this pattern of rust increase is a function of the availability of susceptible tissue, given the presence of inoculum. Within this typical pattern of rust increase, variation among years occurs (Schmidt and Allen 1991) and is assumed to be related primarily to availability of inoculum as conditioned by weather (Davis and Snow 1968, Froelich and Snow 1986). Percentage trees infected at age 5 yr on susceptible family mixtures ranged from 22.8 to 76.1 among locations and averaged 46.5 and 44.2 on susceptible slash and loblolly pine, respectively. This amount of rust incidence was sufficient to compare resistance among family mixtures and locations (Schmidt and Goddard 1976, Sohn *et al.* 1990).

The greater height of loblolly pine compared to slash pine at Statesboro may explain the greater increase in rust incidence on loblolly pine during the 5th yr compared to slash pine at this location and perhaps other locations. For example, Colbert *et al* (1990) found that dry matter partitioning to the crown (foliage and branches) was greater in 4-yr-old loblolly pine when compared to slash pine which partitioned more dry matter into the stem. Dalla-Tea and Jokela (1991) found that loblolly pines (at 6 yr) produced more branches compared to slash pines. The greater number of succulent terminals associated with these branches could provide a greater number and area of potential infection sites on loblolly pine compared to slash pine. Evaluation prior to age 5 yr would have underestimated rust incidence on susceptible loblolly in several locations and changed the rankings of family mixtures at Louisville and Screven. It is not appropriate to define species comparisons from our data which are derived from a small number of select families. Nevertheless our data with similar genetic material at all locations show that rust incidence on slash pine was significantly greater than that on loblolly pine at the most southern location (Perry, FL) and at two locations (Statesboro and McRae) in central GA. Loblolly pine exhibited significantly greater rust incidence than slash pine at the most northern location (Louisville, GA). Overall, rust incidence on slash pine (30.9%) was significantly greater than that (26.4%) on loblolly pine. This difference resulted from significantly less rust incidence on resistant loblolly pine (10.4%) compared with that (16.0%) on resistant slash pine.

Rust incidence on resistant slash pine family mixtures differed significantly among locations. Similar interactions in slash pine were reported by Sohn and Goddard (1979). Our data indicate that resistance in slash pine is closely related to disease hazard, i.e., the resistance/susceptible ratio increased as rust incidence on susceptible material increased. This decrease in relative resistance could result from quantitative or qualitative changes in inoculum potential among locations. Others have reported the "erosion" of fusiform rust resistance with increased amounts of inoculum in naturally inoculated field progeny tests of slash pine (Sohn and Goddard 1979) and

with artificial inoculations of slash and loblolly pines (Matthews *et al.* 1978). In a previous analysis of our data (Hodge *et al.* 1993), the authors concluded that notwithstanding the differences in relative rust resistance in slash pine among locations: 1) best linear predictions of breeding values (White and Hodge 1988) were useful to estimate rust performance across rust incidence, and 2) differences in rust performance among locations were not economically important for these specific mixtures.

Rust incidence on resistant loblolly family mixtures was directly related to rust incidence on susceptible loblolly mixtures, although there were no significant differences in relative resistance (resistance/susceptible ratio) among locations.

Among all locations, there was no significance in the R/S ratios among cultural treatments applied at planting for slash or loblolly pine. This result was likely due to the inconsistent response of pine height to cultural treatments within locations. If the fertilizer and herbicide treatment had consistently increased pine height over that of the control treatment, an associated increase in rust incidence likely would have occurred.

In conclusion, progeny from open-pollinated, rust-resistant slash and loblolly pines planted in six-family mixtures performed well when compared with rust susceptible mixtures at all geographic locations and rust incidences (rust hazard) included in this study. These results suggest that similar resistant family mixtures can be widely planted to reduce rust incidence. However, in slash pine, the relative rust resistance was significantly related to the rust incidence on the susceptible mixtures and relative resistance varied significantly among locations, decreasing with increasing rust hazard. The cause of this decrease in relative resistance of resistant slash pine mixtures is not known, but is directly related to rust hazard (rust incidence) and could be related to pathogenic variability.

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DERIVATION OF HOST AND PATHOGEN GENOTYPES IN THE FUSIFORM RUST PATHOSYSTEM ON SLASH PINE USING A COMPLIMENTARY GENETICS MODEL AND DIALLEL DATA

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Abstract:-- Seedlings from 20, full-sib families of a five-parent slash pine diallel were inoculated using two, single urediniospore-derived cultures of the fusiform rust fungus on two different dates during the 1994 growing season. Presence or absence of fusiform rust galls was recorded for each inoculated seedling at nine months post-inoculation and percent infection levels for each family:inoculum:date combination were calculated. The complementary genetics model normally requires clonal material of either the host or pathogen in order to assign genotypes to identified complementary gene pairs. Diallel data, however, allows these assignments to be made even when both the host and pathogen populations are segregating. For the July data, three pairs of complementary genes are thought to explain the observed percent infection levels among the host families and fungus cultures. The putative genotypes of the fungus cultures WLP-10 and CCA-2 are A_1A_1:A_2a_2:a_3a_3 and A_1a_1:A_2a_2:A_3A_3, respectively. The respective host genotypes for parents 8-7, 9-2, 18-26, 18-62, and 18-27 are R_1R_1:r_3r_3, R_1r_1:r_3r_3, r_1r_1:R_2r_2/r_2r_2:r_3r_3, r_1r_1:r_2r_2/R_2r_2:r_3r_3, and R_1r_1:R_3r_3. The homozygous dominant reaction gene of parent 8-7 at locus 1, complemented by the homozygous dominant avirulence gene in culture WLP-10, is thought to be the reason that families with 8-7 as one of the parents appear to be so "resistant" when challenged with WLP-10. Two additional complementary gene pairs can be hypothesized if the May data is included for families 9-2 x 18-26 and 18-27 x 18-26. The identification of these gene pairs using both the May and July inoculation data suggest the existence of temperature-sensitive genes in this pathosystem. A Chi-square analysis of the July inoculation data using a complementary model with four gene pairs indicate a good fit between expected and observed percentage infection levels. The implications of these findings on rust screening and deployment are discussed.

Keywords: Pinus elliottii, Cronartium quercuum f.sp. fusiforme, complementary genetics, diallel data

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INTRODUCTION

Although phenotypic selection for disease resistance and breeding among recognized resistant trees (Zobel and Talbert 1984) have increased the supply of resistant seedlings, the genetic basis of the host-pathogen interaction in the slash pine (Pinus elliottii Engelm. var. elliottii) : fusiform rust fungus (Cronartium quercuum [Berk.] Miyabe ex Shirai f. sp. fusiforme) pathosystem is not understood despite 37 years of research (Powers 1991). Efforts to characterize this genetic basis have resulted in the hypothesis that the interaction may conform to a complementary genetic system (Griggs and Walkinshaw 1982; Kinloch and Walkinshaw 1990; Nelson et al. 1993). In such a system, the genetics of both the host and the pathogen must be considered (Figure 1). Resistance is considered dominant in the host and avirulence is dominant in the pathogen. Any combination of a particular pair of complementary genes where the complementary gene from one organism is homozygous recessive will result in a high infection type (e.g., a gall). In the case where two or more pairs of complementary genes are present, the gene pair that imparts the low infection type (e.g., no gall) is epistatic to all other gene pairs.

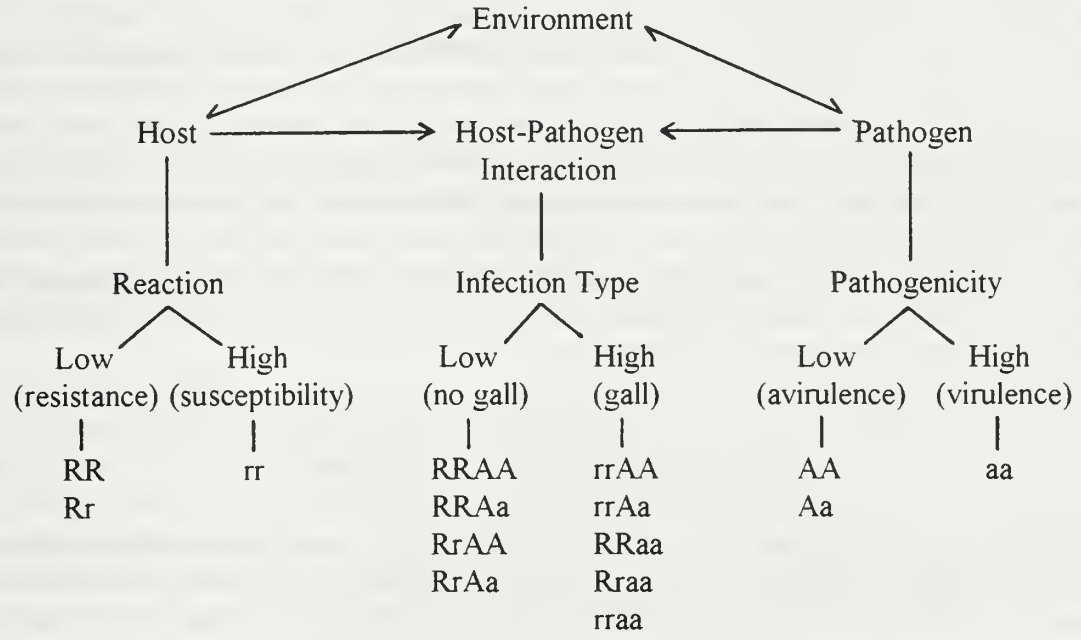

Figure 1. Simplified schematic of the complementary genetics model.

To demonstrate the operation of a complementary genetic system, the ideal model would involve two symbionts in which classical diploid inheritance occurs and that can be propagated as clones (Loegering 1984). This would make it possible to observe the phenotype of all possible combinations of the F_2 individuals of both symbionts. Of the models worked with to date, the one that best approximates the ideal is flax rust. The pathogen, Malampsora lini (Pers.) Lev., has

diploid inheritance and the uredial cultures are clones. It is genetically stable and is easily maintained. Flax, Linum usitatissimum L., is self-pollinated and has normal diploid inheritance. While it is difficult to propagate as a clone, sequential inoculation of a given plant with different cultures of the pathogen is possible.

The fusiform rust pathosystem on slash pine presents some challenges to the ideal model. First and foremost, the non-repeating spermatial-aecial stage of the fungus is the one that causes the economic damage we wish to control. As a result, specific cultures of the fungus cannot be clonally propagated. The basidiospores infecting the slash pine have been shown to be haploid products of meiosis and hence represent a segregating population (Doudrick et al. 1993a,b). Efforts have been made to develop in vitro inoculation techniques using single genotype haploid cultures of the fungus, but they have been largely unsuccessful (Frampton 1984; Hu 1990). Significant problems with the host include the long time it takes to generate F_1 and F_2 populations (compared to agronomic species), and the inability to clonally propagate large numbers of selected host genotypes. Rooted cuttings are currently the most efficient means of vegetative propagation. Rooting success however, is still very much dependent upon the genotype of the individual. Hence, it is difficult to reliably produce the genetic array needed.

One possible way to circumvent these problems is to study the genetics of one member of the relationship in detail. Moseman (1966) working with barley mildew (Hordeum vulgare L.:Erysiphe graminis D.C. f.sp. hordei Em. Marchal) has clearly demonstrated that the genetics of one member of the relationship can be determined by studying the genetics of the other member. A recent study conducted at the Southern Institute of Forest Genetics however, suggests that by inoculating families of slash pine from a diallel mating scheme using single-urediniospore derived cultures of the rust fungus, the problem of segregating host and pathogen populations can be mitigated. The objective of this paper is to further develop hypothetical genotypes for individual parents of slash pine and single-urediniospore derived cultures of the rust fungus using a complementary genetics model and this diallel data.

MATERIALS AND METHODS

Host seedlings. Five, first-generation selections of P.e. elliottii were control-pollinated in a full-diallel crossing design. All five selections were chosen from the U. S. Forest Service's Harrison Experimental Forest in Harrison County, MS (Griggs et al. 1983, unpublished USFS SRS Study Plan No. 3.48 available on request). Previous research has classified these selections using bulk inocula of the rust fungus as follows: parent 8-7, resistant; parents 9-2 and 18-27, moderately resistant; and parents 18-26 and 18-62, susceptible (Jewell and Mallett 1967; Snow et al. 1975). Twenty of the 25 full-sib families (selfs excluded due to insufficient seed) were propagated from seed (Doudrick et al. 1996). Seed germination was staggered so that the seedlings were eight weeks old at the time of each inoculation series.

Pathogen cultures. Two single urediniospore-derived cultures of C.q. fusiforme were used in deriving the hypothetical genotypes: CCA-2, WLP-10 (G. Snow. 1984, unpublished USFS SRS

Study Plan No. 20.55 available upon request. Both cultures originated from aeciospore collections made in 1984: CCA-2 on planted Livingston Parish (LP) loblolly pine (P. taeda) in Madison County, FL; WLP-10 on planted LP loblolly pine in Livingston Parish, LA. Single urediniospore-derived cultures were then developed from the aeciospore collections (Doudrick et al. 1993b; Powers 1980). This analysis is part of a larger study that included two additional cultures of the rust fungus. They have been excluded for brevity and because they did not provide any additional information.

Artificial inoculations. Seedlings of each full-sib family were inoculated using each of the four pathogen cultures on two different dates, May 23 and July 12, in 1994. For each inoculation date and pathogen culture, 16 to 80 seedlings from each family were randomly divided into four replications and inoculated. In each replication, the families were inoculated in random order using a forced-air apparatus (Snow and Kais 1972). The succulent terminal shoot of each seedling was inoculated at a density of 12 to 18 spores per mm^2, and the inoculum density was verified after every tenth seedling inoculated.

After inoculation, the infected seedlings were incubated in the dark at 20 to 22 °C and 100% relative humidity for 24 hours. After incubation, the seedlings were returned to the greenhouse and grown under an 18-hour-light photoperiod provided by 1,000-W metal halide lamps. Two weeks after inoculation and every second week thereafter, the seedlings were fertilized using 20-20-20 (200 ppm N).

Data collection and initial analysis. The presence or absence of fusiform rust galls on the seedlings was recorded nine months after inoculation. A non-galled seedling received a score of 0, while a galled seedling received a score of 1. The percentage infection was then determined for each replication within a given full-sib family x inoculation date x inoculum treatment combination.

The data were initially analyzed using the SAS general linear model procedure (GLM, 6th ed., SAS Institute Inc., Cary, NC) to test for significant effects. Because percentage infection data are based upon a binomial response and some data points lie outside the stable variance range of 30 to 70 percent, all data were transformed to the arc sin of the square root of the percentage infection (Anderson 1974). Family, inoculum, and inoculation date were all considered to be fixed effects. Reciprocal cross effects were treated as a nested effect within family and considered fixed as well. The replication effect was nested within inoculation date and regarded to be random.

Derivation of hypothetical genotypes. Within a particular inoculation date, a significant family x inoculum interaction effect suggests that differential interactions exist between two or more fungus cultures and two or more host families. These differential interactions are equivalent to the interactions that were developed by Loegering and Burton (1974) and suggest that two pairs of complimentary genes could be operating in the host and pathogen. For each inoculation date, differential interactions were visually identified by sequentially stepping through the table one pair of host families at a time across all possible pairs of the inocula. Once a differential interaction was found involving two full-sib families and two fungus cultures, where percentage infection

levels either increased or decreased by at least 12 percent (≥ 2xSD), pairwise comparisons between the four proportions were made in order to test for statistical significance (Neter et al. 1982).

Having identified the potential number of complementary gene pairs, the percentage infection data were examined for a unique combination of host pedigree, fungus culture, and inoculation date that could serve as a starting point for assigning hypothetical genotypes. The process then involved a series of repetitive iterations, using deductive reasoning within the constraints of the complementary genetics model. In the interest of presenting the concepts of the complementary genetics model in a clear and concise format, we will forego the use of the nomenclature that has been proposed by Doudrick et al. (1994). Subsequent manuscripts will include proper nomenclature.

RESULTS

Analysis of variance revealed no significant reciprocal cross effects (P=0.1118), therefore, forward and reciprocal cross data were pooled. Likewise, the replication effect was not significant (P=0.0901). Pooling the replications for a given family:inoculum:date combination increased the average sample size from 9 to 72 and hence greatly increased the power of estimating percentage infection levels. Family, inoculum, and family x inoculum effects were all significant (P=0.0001) and accounted for 49 percent of the observed variation. These data suggest that infection type reversals exist. While the date main effect was not significant (P=0.0833), the two-way interactions involving date implied that date did play some role in the development of disease symptoms.

Differential interactions. Percentage infection data for all family:inoculum:date combinations are given in Table 1. Several significant (P=0.0500) differential interactions among particular pairs of host families and fungus cultures were identified in both the May and July inoculation series. These interactions are delineated in Table 1 by lower- and upper-case letters for the May and July inoculation dates, respectively. Four significant differential interactions were found among the July inoculations. All four differential interactions shared the common family, 18-27 x 18-62. The other host families involved were 8-7 x 9-2, 8-7 x 18-26, 8-7 x 18-62, and 9-2 x 18-26. Only two differential interactions were found among the May inoculations.

Inoculation date effects. Changes in percentage infection data between the May and July inoculations were observed in several families for the two fungus cultures (Table 1). When challenged using the WLP-10 inoculum, all host families having 8-7 as one of the parents exhibited a significant reduction in infection levels from the May to July inoculations. For CCA-2, the percentage infection increased from May to July regardless of host pedigree.

Three significant differential interactions involving inoculation dates were found by looking either within a given fungus culture for infection type reversal among two host families, or within a given host family for infection type reversal between the fungus cultures. These interactions are

identified in Table 1 by superscript Roman numerals. The first two interactions involved culture WLP-10 and host families 8-7 x 9-2, 18-27 x 18-26, and 9-2 x 18-26. The third differential inoculation date interaction involved both fungus cultures and host family 9-2 x 18-26.

Table 1. Percentage infection levels (percent ± sd) of ten, full-sib families of slash pine inoculated using single urediniospore-derived cultures CCA-2 and WLP-10 of the fusiform rust fungus on May 23 and July 12, 1994. Average sample size per family:inoculum:date was 72 seedlings.

	Culture			
	CCA-2		WLP-10	
Family	May	July	May	July
8-7 x 18-27	20 ± 5	30 ± 5	11 ± 4	1 ± 1
8-7 x 9-2	39 ± 5	57 ± 6^{B}	16 ± 4	3 ± 2^{B}
8-7 x 18-26	44 ± 7	50 ± 6^{A}	40 ± 7^{I}	4 ± $2^{A.I}$
8-7 x 18-62	50 ± $6^{a.b}$	59 ± 6^{C}	20 ± $5^{a.b}$	3 ± 2^{C}
18-27 x 9-2	16 ± 5	33 ± 6	18 ± 5	21 ± 5
18-27 x 18-26	23 ± 6	44 ± 7	22 ± $6^{I.II}$	50 ± $7^{I.II}$
18-27 x 18-62	23 ± 5	38 ± $5^{A.B.C.D}$	29 ± 5	55 ± $6^{A.B.C.D}$
9-2 x 18-26	34 ± $6^{a.III}$	71 ± $6^{D.III}$	52 ± $6^{a.II.III}$	30 ± $5^{D.II.III}$
9-2 x 18-62	36 ± 5	80 ± 5	36 ± 5	44 ± 6
18-26 x 18-62	38 ± 5^{b}	70 ± 6	65 ± 5^{b}	70 ± 5

Note: Differential host and rust fungus pairs are identified by matching lower-case letters for the May inoculation, upper-case letters for the July inoculation, and Roman numerals for between inoculation dates.

Derivation of hypothetical genotypes. If one looks at the July inoculation, all families having 8-7 as a parent that were inoculated with the fungus culture WLP-10 exhibited extremely low levels of infection. Since the basidiospore is haploid, and by definition of the complementary genetics model, culture WLP-10 must be homozygous dominant for avirulence (A_1A_1) at this first locus. On the host side, one of the parents must be homozygous dominant for resistance (R_1R_1) at this locus. Otherwise, if both parents were heterozygous, R_1r_1, then one should have observed an infection level of 25%. The odds are very low that four unique parents would all be R_1R_1; therefore, we conclude the common parent, 8-7, is R_1R_1. Parents 9-2, 18-26, 18-62, and 18-27 could either be R_1r_1 or r_1r_1.

As one proceeds down through the pedigrees for the July inoculation, no pattern emerges that is helpful in assigning host genotypes at locus 1 until family 18-26 x 18-62 at 70% infection is encountered. The only way one can observe an infection level greater than 50% is to assume that both the host gene and pathogen gene at that complementary locus are segregating. The pathogen's genotype must be Aa. Two scenarios can exist for the complementary host gene.

Either both parents are heterozygous Rr, or one parent is heterozygous Rr and the other parent is homozygous recessive rr. The former case would result in a 38% infection level and the latter case would generate a 75% infection level. Given the observed percentage infection of 70%, we conclude that one parent is heterozygous and the other parent is homozygous recessive for this gene pair.

But, we have already made the assumption that WLP-10 is A_1A_1. Therefore, there must be another pair of complementary genes that are segregating. The putative genotype of the fungus culture WLP-10 is now A_1A_1,A_2a_2, parent 18-26 is $r_1r_1,R_2r_2/r_2r_2$, and parent 18-62 is $r_1r_1,R_2r_2/r_2r_2$. At this time, we do not know which parent is heterozygous for the second complementary host gene. Furthermore, for this second gene pair to be expressed, by definition of the model, both host parents 18-26 and 18-62 must be homozygous recessive at the first locus.

Given a two-gene model and the hypothetical genotypes of the fungus culture WLP-10 and parents 18-26 and 18-62, we need to develop the hypothetical genotypes of the remaining three parents before we proceed. The fact that a complementary gene pair that imparts a low infection type is epistatic to all other gene pairs and that parent 8-7 is homozygous dominant R_1R_1 prevents us from verifying that parent 8-7 possesses the second reaction gene.

The remaining two parents, 18-27 and 9-2, are also thought to be lacking the second reaction gene. If both of these parents are assumed to be R_1r_1, then when they are crossed with either 18-26 or 18-62 (r_1r_1) the expected percentage infection among the progeny inoculated with fungus culture WLP-10 should be 50%. When 18-27 and 9-2 are crossed, the expected percentage infection would be 25%. With the exception of family 9-2 x 18-26, the observed infection levels corresponded to the expected values.

Let us further develop these hypothetical genotypes by incorporating the July inoculation data using the fungus culture CCA-2. The complementary gene pair at locus 1 can explain the observed infection levels for families 8-7 x 9-2, 8-7 x 18-26, and 8-7 x 18-62, if one assumes that instead of the fungus culture CCA-2 being homozygous dominant for avirulence it is in fact heterozygous A_1a_1. In all of the cases, a low infection level would occur 50% of the time. If this assumption is made, then the only way to obtain a 30% infection rate in family 8-7 x 18-27 would be to invoke a third locus. The pathogen would be homozygous for avirulence, A_3A_3, and the host genotypes for this family would be R_3r_3 and r_3r_3, segregating in a 1:1 ratio. Parent 8-7 is hypothesized to be the homozygous recessive parent. Again, the key point to remember is that the gene pair invoking the low infection type is epistatic to all other gene pairs. In this family, the resulting offspring have the genotype R_{1_1} at locus 1. Since we have assumed the pathogen is heterozygous at locus 1, we would expect 50% infection. However, the epistatic effect of locus 3 would result in 50% of the 50% infected if only locus 1 were operating to be resistant; therefore, we would expect an overall infection of 25%.

Given that parent 8-7 is hypothesized to be r_3r_3, parents 9-2, 18-26, and 18-62 must also be r_3r_3 in order for this locus not to be epistatic to locus 1 and yield expected infection levels comparable to the observed values. The theoretical infection level of 31.25% in family 18-27 (R_1R_1,R_3r_3) x 9-2

(R_1r_1,r_3r_3) is also a result of this epistasis and is comparable to the observed infection level. Likewise, similar epistatic effects are hypothesized for the infection levels observed in families 18-27 (R_1r_1,R_3r_3) x 18-62 (r_1r_1,r_3r_3), and 18-27 (R_1r_1,R_3r_3) x 18-26 (r_1r_1,r_3r_3). Family 9-2 ($R_1r_1r_3r_3$) x 18-26 ($r_1r_1r_3r_3$) and family 9-2 ($R_1r_1r_3r_3$) x 18-62 ($r_1r_1r_3r_3$) would both be expected to exhibit infection levels of 75% because the segregation is in both symbionts at the first gene pair. As in the case of family 18-26 x 18-62 inoculated using culture WLP-10, we hypothesize that the second complementary gene pair is responsible for the observed infection levels of 70% when this family is inoculated with culture CCA-2.

At this point, the theoretical expectations of only one family, 9-2 x 18-26, does not fit the observed infection levels. If we now include the May inoculation data however, a possible explanation comes to light. As noted earlier, a date-dependent differential interaction was observed within the WLP-10 culture for families 18-27 x 18-26 and 9-2 x 18-26. By invoking a fourth complementary gene pair that is homozygous avirulent, A_4A_4, in either May or July on the pathogen side and segregating in a 1:1 ratio for R_4r_4:r_4r_4 on the host side, the theoretical infection level for the family 9-2 (R_1r_1,R_4R_4) x 18-26 (r_1r_1,r_4r_4) becomes 25% and now is comparable to the observed 30% infection level.

Table 2 summarizes the observed and expected percentage infection levels for the July inoculation. A Chi-square analysis to test the goodness-of-fit of this theoretical model to the observed percentage infection levels, yielded a test statistic of $\chi^2 = 6.63$. Since the test statistic is less than the critical $\chi^2_{(19,.05)}$ of 30.14 we conclude this complementary genetics model of four gene pairs adequately describes the observed results .

Table 2. Observed (OBS) and expected (EXP) percentage infection levels of ten, full-sib families of slash pine inoculated using single urediniospore-derived cultures of the fusiform rust fungus, CCA-2 and WLP-10, on July 12, 1994.

	Culture			
	CCA-2		WLP-10	
Family	OBS	EXP	OBS	EXP
8-7 x 18-27	30	25	1	0
8-7 x 9-2	57	50	3	0
8-7 x 18-26	50	50	4	0
8-7 x 18-62	59	50	3	0
18-27 x 9-2	33	31.25	21	25
18-27 x 18-26	44	37.50	50	50
18-27 x 18-62	38	37.5	55	50
9-2 x 18-26	71	75	30	25
9-2 x 18-62	80	75	44	50
18-26 x 18-62	70	75	70	75

DISCUSSION

The results of this study clearly demonstrate the power of using diallel data not only to study the genetics of the host, but the pathogen as well in the fusiform rust pathosystem on slash pine. Clonal material of slash pine would have allowed us to sort the results into union phenotypes and derive hypothetical genotypes similar to Nelson et al. (1993). The diallel data however, did help mitigate the observed segregation. Even the most complex percentage infection levels could be explained using the complementary genetics model and the diallel data.

It was hypothesized that two parents, 9-2 and 18-27, did not possess the reaction gene associated with the second complementary locus. As this is a coevolved pathosystem, one would not expect every host genotype to possess the corresponding reaction gene for every complementary pair that exists in the pathosystem. Not all individuals will possess all the genes for every complementary pair, nor could any individual possess all alleles at these genes. For the cereal rust system, resistance genes have been shown to be members of small gene families (Loegering and Powers 1962). Mutations, insertions, deletions, and duplications in both the host and pathogen genome during sexual reproduction, as well as random drift, all drive the genetics of the pathosystem.

These data also show the potential pitfall of using bulk inocula to screen host genotypes. For example, if family 8-7 x 9-2 were screened with even a simple bulk inocula such as CCA-2 and WLP-10, one would falsely conclude that this family exhibited good "resistance" to the fungus. If this family is then deployed in an area where CCA-2 is the predominating inoculum however, the apparent resistance immediately would break down.

Another potential hazard in the screening process is that these evaluations might be temperature dependent. If temperature is important, then screening evaluations might not correlate well to field performance. For example, the good "resistance" based on a July screening of those families having 8-7 as a parent might easily appear to have broken down when those outplanted individuals are exposed to the same fungus in May. When in fact, other gene pairs are interacting and the "July interaction" would still be observed under appropriate environmental conditions.

It could be argued that the inoculation date effect is an artifact of the artificial inoculations. The warmer day/night temperatures of July may have so affected the physiology of either the host seedlings, or the fungus cultures, that the disease syndrome failed to develop. This hypothesis could account for the reduction in the July percentage infection levels for those pedigrees having 8-7 as a parent when they were challenged with fungus culture WLP-10. This host physiological resistance does not explain, however, the ability of those same families to exhibit higher percentage infection levels when inoculated using culture CCA-2 in July.

The environment is known to profoundly affect the expression of infection types (Waterhouse 1929). Temperature-sensitive genes in both the host and pathogen have been identified in the wheat:stem rust pathosystem (Knott and Anderson 1956; Loegering and Geis 1957).

Furthermore, it was demonstrated that these temperature-sensitive genes adhere to the complementary genetic model (Loegering 1968). Similar temperature-sensitive genes could be at play in the differential reactions observed in this study.

A more definitive study that involves clonal lines of these host pedigrees and seven, single urediniospore-derived cultures of the rust fungus currently is being established. The results of the current study will enable us to focus our efforts on those host families and fungus cultures that were involved in differential interactions. In addition, the galled trees from the current study have been maintained and specific crosses of the fungus will be initiated this fall. These combined efforts will provide us with the opportunity to advance the knowledge on complementary genetic interactions in fusiform rust disease.

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GENETIC AND RECIPROCAL EFFECTS ON FIRST-YEAR GROWTH AND LEAF AREA DEVELOPMENT OF SYCAMORE SEEDLINGS

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Abstract:-Seedlings from control-pollinated crosses of American sycamore (*Platanus occidentalis L.*) were studied for genetic and reciprocal effects on seedling growth and leaf area development during the first growing season in a nursery. Reciprocal effects accounted for most of the variation among families in seedling heights during the first month, but these effects then disappeared. After a dry period during the fourth month (August), reciprocal effects were detected for stem volume and for number and leaf area of live leaves per seedling. Genetic effects were responsible for family variation detected in (1) seedling height and diameter during the other portions of the growing season, (2) seedling stem volume at the end of the season, and (3) leaf area development before the August dry period. Genetic differences in seedling size at the end of the first growing season were related to differences in leaf area expansion early in the growing season ($r = 0.97$). The fastest-growing cross (pair of reciprocals) had the largest leaf area per seedling by three months (July) after the seeds were planted, and it maintained the greatest height, diameter, and stem volume throughout the growing season.

Keywords: Reciprocal crosses, Common environmental effects, Family variation, Seedling growth, Leaf expansion, *Platanus occidentalis L.*

INTRODUCTION

Early selection for genetic improvement of woody crops has recently received considerable attention from tree breeders, because it may be used to shorten generation intervals and reduce costs of progeny tests. Increased genetic gain per unit time may be achieved from selection of juvenile traits for improvement of mature traits, but this requires high juvenile-mature correlations and a reasonable heritability for each trait (McKinley and Lowe 1986). Strategies of early selection and breeding have been proposed for increasing productivity of sycamore (*Platanus occidentalis L.*) in short-rotation energy plantations (Land 1981).

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Seedling characteristics are juvenile traits and may be subject to maternal (non-nuclear genetic) and common environmental effects of the mother tree. Differences between progenies of reciprocal crosses (sex roles of parents reversed) provide a measure of these effects. Maternal and common environmental effects cause resemblances among offspring from the same mother tree that are not chromosomally inherited, and the effects can increase variation among different mother-tree families (Falconer 1989). Such effects can give overestimates of heritability and thereby provide erroneous conclusions about expected genetic gains from early selection. Several studies have reported maternal or environmental effects in forest tree species. Perry (1976) and Barnett (1991) have pointed out that maternal effects in coniferous species influenced seed size and germination rate, and early seedling performance. Large seeds of loblolly pine germinate more quickly and produce larger germinants than small ones (Dunlap and Barnett 1983). Robinson and van Buijtenen (1979) found a positive relationship between seed weight and 15-year tree height of loblolly pine in a progeny test. Khalil (1981) reported that seed weight of *Picea glauca* was positively correlated with annual terminal shoot growth of young trees. Land *et al.* (1989) conducted a germination test with control-pollinated seed of sycamore. They found significant differences in seed length and weight, rates of germination, and germination values between reciprocal crosses. However, no studies have been conducted to quantify the relative magnitudes of genetic and reciprocal influences on seedling performance of sycamore. Information on how genetic and reciprocal effects influence juvenile traits is needed for tree improvement programs of quasi-juvenile traits in short-rotation energy plantations. The objectives of the present research were to: (1) determine monthly change and genetic variation in height, root-collar diameter, stem volume, and leaf characteristics of sycamore seedlings during the first growing season under nursery conditions, and (2) evaluate the relative importance of reciprocal effects on seedling growth, leaf area, and root characteristics.

MATERIALS AND METHODS

Plant Material and Experimental Design. A single-pair mating scheme, with reciprocals, was used among 12 sycamore trees to produce 12 control-pollinated families (six pairs and two reciprocals per pair). The six pairs included L108 x G106 (L x G), T205 x T108 (T x T), H205 x S210 (H x S), B104 x B110 (B x B), B209 x N109 (B x N), and K110 x C204 (K x C). The 12 parent trees came from seed sources in Alabama, Arkansas, Louisiana, and Mississippi and were represented by grafts in a clonal orchard at Mississippi State University (MSU) in northeast Mississippi (33°27' N. latitude and 88°45' W. longitude). Four seeds per container from each of the 12 families were planted on April 14, 1989 in containers (15 cm x 15 cm x 40 cm deep) filled with a 1:1 mixture of coarse sand and a local fine sandy loam soil at the MSU outdoor nursery. Germinants were thinned to the tallest seedling per container on June 12 (three months after seeds were planted). In addition, the germinants were fertilized with 13-13-13 slow-release fertilizer and grown for eight months in the nursery with daily morning irrigations until mid-October.

The experimental design in the nursery was a randomized complete block with four replications. Each family plot within a replication consisted of a single row of eight seedlings (eight containers) and therefore an entire replication had a total of 96 seedlings. The seedling spacing was 15 cm by 15 cm.

Nursery Measurements. Germinant heights were recorded at 7, 14, 22, 28, and 56 days after seeds were planted. Measurements of heights and root-collar diameters continued on a monthly basis from three-months (July) to eight-months (December) seedling age. Number of branches, numbers of living and dead leaves on each branch and on the main stem, and leaf widths of each live leaf were taken for each seedling at two-week intervals from July to mid-November.

The eight-month-old seedlings were removed from the containers and measured for heights and root-collar diameters in mid-December. The roots were carefully washed and measured for (1) number and length of tap roots per seedling and (2) numbers of lateral roots in each of three size classes per seedling. The three size classes were based on lateral-root diameter adjacent to the connection with the tap root: (1) "fine" roots were less than 2.0 mm diameter, (2) "medium" roots were between 2.0 and 5.0 mm diameter, and (3) "large" roots were greater than 5.0 mm diameter. Root volume per seedling was measured by water displacement in a graduated cylinder and recorded separately for tap roots and each of the three sizes of lateral roots.

Data Analyses. Leaf areas of seedlings were calculated from leaf widths with the following equation:

$$\text{Ln (leaf area)} = 1.8 \times \text{Ln (leaf width)} \qquad (1)$$

where Ln is the natural logarithm.

Equation (1) was developed using SAS regression analysis procedures (SAS Institute Inc. 1985) on a data set of 200 sample leaves that were measured for leaf widths and actual areas with a LI-COR LI-3000 portable leaf area meter (LI-COR, Inc., Lincoln, NE, USA) (Tang and Land 1996). The fit of these data to the equation was almost perfect ($r = 0.99$). Seedling stem volume (volume index) was estimated as one-third of seedling root-collar basal area multiplied by seedling height (the equation for volume of a cone).

Table 1. Format of analyses of variance used to study genetic and reciprocal effects on seedling growth and leaf area development of sycamore.

Source of Variation	d.f.	Expected Mean Squares[a]
Replications (R)	3	$(1/h)\sigma^2_w+\sigma^2_{RC(P)}+2\sigma^2_{RP}+12\sigma^2_R$
Pairs (P)	5	$(1/h)\sigma^2_w+\sigma^2_{RC(P)}+2\sigma^2_{RP}+4\sigma^2_{C(P)}+8\sigma^2_P$
Crosses within Pairs (C:P)	6	$(1/h)\sigma^2_w+\sigma^2_{RC(P)}+4\sigma^2_{C(P)}$
R x P	15	$(1/h)\sigma^2_w+\sigma^2_{RC(P)}+2\sigma^2_{RP}$
R x C:P	18	$(1/h)\sigma^2_w+\sigma^2_{RC(P)}$
Within plots	336	σ^2_w

[a] (1) All effects assumed random, and σ^2 = variance component.
(2) The letter "h" is the harmonic mean number of living seedlings per block.

Analyses of variance with a format given in Table 1 were conducted using GLM procedures of SAS (SAS Institute Inc. 1985). Variation within plots was calculated on an individual seedling basis, whereas the analysis of other sources of variation was based on plot means due to the imbalanced data caused by differences in seedling survival among family plots within replications. F-tests were constructed and computed from the expected mean squares in Table 1 (Snedecor and Cochran 1980) to determine differences among six pairs of crosses (i.e., genetic effects) in seedling growth, leaf area expansion, and root characteristics. The approximate degrees of freedom were calculated for the F-tests of genetic differences using a Satterthwaite's procedure. Reciprocal effects were indicated by differences between two reciprocal crosses within each pair. Genetic and reciprocal effects were considered significant if F-test statistics revealed that the respective sources of variation had probability levels of 0.05 or less. When genetic and/or reciprocal effects were significant, Duncan's multiple range test (Steel and Torrie 1980) was conducted to test the ranked pair and cross means for significant differences at $P \leq 0.05$.

RESULTS AND DISCUSSION

Monthly Trends in Seedling Growth and Leaf Area Development. Maximum rate of height growth occurred in July and was 26.1 cm for that month, while the greatest monthly diameter growth rate was 1.8 mm in August (Table 2). Stem volume had the largest monthly increment (6.5 cm^3) in October, but growth had almost ceased by mid November. Eight-month-old seedlings (end of the growing season) averaged 90 percent in survival, 64 cm in height, 8.9 mm in root-collar diameter, 16.8 cm^3 in stem volume, and 36 lateral roots.

Table 2. Average monthly rainfall, leaf area, and growth of sycamore seedlings during the 1989 growing season[a].

Month	Average Rainfall (mm)	Current Living Leaves		Monthly Growth Increment		
		Leaf area ($cm^2 \cdot seedling^{-1}$)	No. leaves ($seedling^{-1}$)	Height (cm)	Diameter (mm)	Stem volume (cm^3)
May	180	---	---	0.2 (0.0)	---	---
June	262	---	---	3.6 (0.2)	---	---
July	160	858 (51)	10.4 (0.7)	26.1 (1.1)	---	---
August	61	658 (48)	9.5 (0.7)	20.2 (0.3)	1.8 (0.0)	4.0 (0.2)
September	190	1060 (77)	14.5 (0.9)	10.8 (0.2)	1.2 (0.0)	4.4 (0.4)
October	10	516 (72)	7.8 (0.8)	2.8 (0.3)	1.6 (0.1)	6.5 (0.5)
November	159	213 (48)	2.6 (0.4)	0.0 (0.0)	0.1 (0.0)	0.2 (0.0)
December	124	---	---	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

[a] The number in parentheses is the standard error of the mean.

Live leaves on main stems reached a maximum size of 118 $cm^2 \cdot leaf^{-1}$ in July and accounted for 74 to 88 percent of the total leaf area per seedling through the end of the growing

season. Branch live leaves represented the remaining total during that period and became a full size of 29 $cm^2 \cdot leaf^{-1}$ in September. The greatest leaf area per seedling was 1060 cm^2 and occurred in September, because (1) both stem and branch leaves had been fully expanded at that time, and (2) the largest number of live leaves per seedling (14.5 leaves) was reached in that month (Table 2). However, the month of greatest leaf area may vary from year to year, depending on timing of drought periods. In the present study the leaf area declined by 27 percent from July to August during a dry period, when the seedlings lost seven percent of leaves on the stem, 24 percent of the branches that bore leaves, and 20 percent of leaves on the remaining branches. The losses of leaf area were followed by a 33-percent reduction in diameter growth increment during the subsequent period from mid August to early September. Number of live leaves and leaf area increased with increased rainfall in September (Table 2). Consequently, diameter growth increased in October, and stem volume growth reached a maximum rate at that time.

Genetic and Reciprocal Effects. *(1) Seedling growth* - Genetic variance components (among six pairs) were significant and larger than reciprocal components (between reciprocal crosses within each pair) for seedling height, diameter, and stem volume starting three months (July) after seeds were planted (Table 3). The only exception to this trend was for diameter in October and stem volume in September and October. Genetic effects, although not significant, also accounted for 66, 73, and 100 percent of the total family variation (among all 12 crosses) in numbers of fine, medium, and large lateral roots in December. Pair L x G had larger seedling height, diameter, and stem volume than other pairs at the end of the growing season (Figures 1a, 1b, and 1c).

Table 3. Relative sizes of estimated variance components for height, diameter, stem volume, and number of lateral roots of sycamore seedlings as measured on a given date during the 1989 growing season.

Measurement Date	Relative sizes of variance components as % of the family component[a]											
	Height		Diameter		Stem volume		Fine roots		Medium roots		Large roots	
	P[b]	C:P	P	C:P	P	C:P	P	C:P	P	C:P	P	C:P
	%											
April 14	0.0	100**	—	—	—	—	—	—	—	—	—	—
April 28	0.0	100**	—	—	—	—	—	—	—	—	—	—
May 6	0.0	100**	—	—	—	—	—	—	—	—	—	—
May 12	17.3	82.7**	—	—	—	—	—	—	—	—	—	—
June 9	35.9	64.1	—	—	—	—	—	—	—	—	—	—
July 14	97.0*	3.0	100**	0.0	100**	0.0	—	—	—	—	—	—
August 11	100*	0.0	100**	0.0	100**	0.0	—	—	—	—	—	—
September 15	100*	0.0	81.3*	18.7	51.3	48.7	—	—	—	—	—	—
October 13	100*	0.0	53.4	46.6	14.0	86.0*	—	—	—	—	—	—
December 13	100*	0.0	82.4*	17.6	54.7**	45.3	65.8	34.2	73.3	26.7	100	0.0

[a] * = Significant at $P \leq 0.05$ and ** = Significant at $P \leq 0.01$.

[b] P = Variance component among pairs, and C:P = Variance component between two reciprocal crosses within pairs. Negative estimates were given a value of zero.

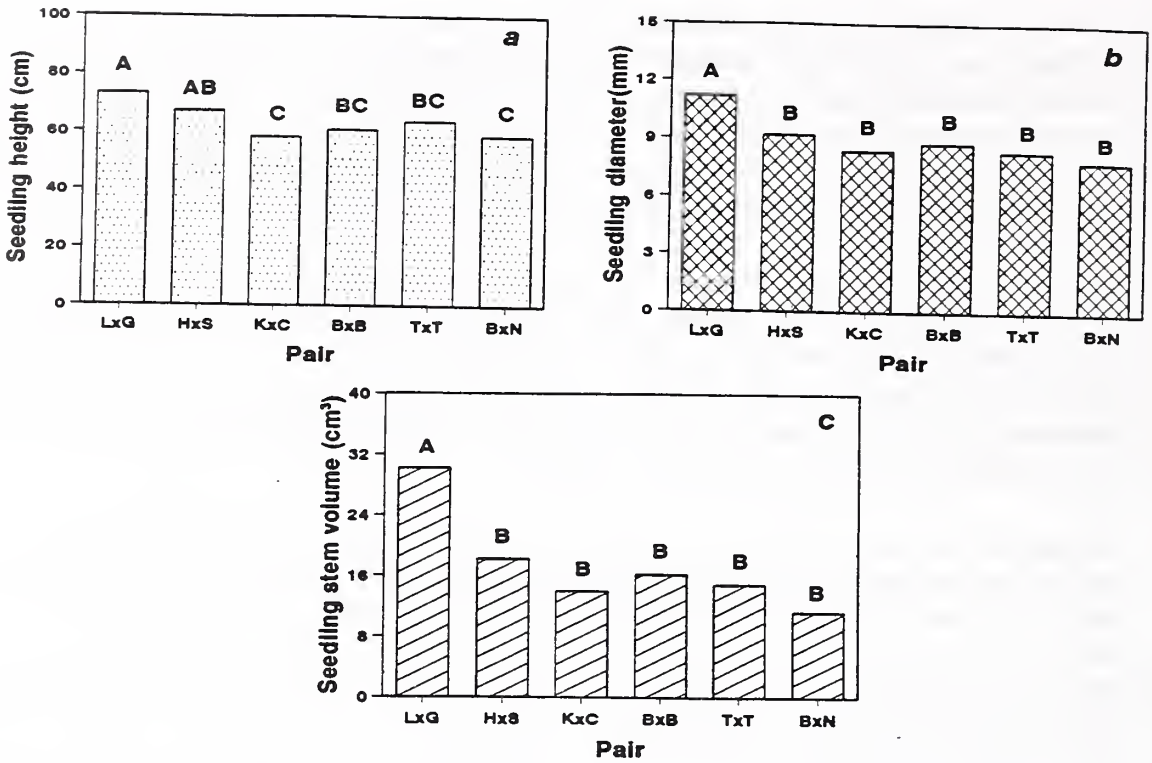

Figure 1. Mean comparisons of height (a), diameter (b), and stem volume (c) in December for six pairs of sycamore seedlings (abbreviations of the pairs are given in the Materials and Methods). Bars not labeled by the same letters differ at $P \leq 0.05$ (experimentwise error at $P \leq 0.23$).

Reciprocal effects influenced seedling heights during the first month after planting and were responsible for 83 to 100 percent of the family variation during that period (Table 3). These effects decreased and eventually disappeared for seedling heights by mid July (three months after planting), but significantly affected stem volume in October following the August dry period. Reciprocal effects had little influence (zero to 34 percent of the family variation) on numbers of fine, medium, and large lateral roots at the end of the growing season (Table 3).

The reciprocal effects on one-month-old seedling height were due to significant reciprocal-cross differences in April and May for two pairs (L x G and B x B). Crosses L108 x G106 and B104 x B110 had larger seedling heights than did their reciprocal crosses during that period. Cross L108 x G106 also produced a significantly larger stem volume by October than the reciprocal G106 x L108 (33.4 cm^3 versus 16.9 cm^3). The two crosses showed greater seed weights and nursery germination values and germination peak values (Land *et al.* 1989). For instance, cross L108 x G106 had significantly lower full seed weight and empty seed weight than the reciprocal G106 x L108 (2.9 $mg \cdot seed^{-1}$ versus 4.2 $mg \cdot seed^{-1}$, $P \leq 0.01$, and 1.9 versus 3.3 $mg \cdot seed^{-1}$, $P \leq 0.01$, respectively). Clone G106 exhibited poor vigor during the year of pollination and seed development, and it died during the year after seed collection as an apparent result of graft incompatibility. This poor vigor may have provided a poor common maternal environment for the seeds of cross G106 x L108. This study indicates that reciprocal-cross differences in seedling height and stem volume may

result from reciprocal effects on seed sizes and germination rates. Similar results have been reported from several studies in pine species (Perry 1976, Dunlap and Barnett 1983, Barnett 1991). However, relationships between mother-tree vigor, seed quality, and reciprocal effects on first-year sycamore seedling growth and development cannot be proven from the present study. The observed coincidence of graft incompatibility and large reciprocal effects suggests that further studies of the relationships are needed.

(2) Leaf area development - F-tests and estimates of variance components indicated that genetic effects (variation among six pairs) accounted for all of the family variation in number of live leaves and total leaf area per seedling through August 7 (nearly four months after planting) (Table 4). Differences among pairs were also significant for leaf areas on main stems and branches during that period (Figures 2a and 2b). Genetic differences in total leaf area per seedling in July were linearly correlated with differences in seedling stem volume in December ($r = 0.97$) (Figure 3). The greater leaf area per seedling the pairs produced, the larger stem volume they had. Rapid leaf area expansion early in the growing season is apparently of direct importance to first-year seedling size, which is comparable to results found for *Populus* hybrids (Isebrands and Nelson 1982, Ridge *et al.* 1986, Michael *et al.* 1988).

Table 4. Relative sizes of estimated variance components for leaf area of sycamore seedlings during the 1989 growing season.

Measurement Date	Relative sizes of variance components as % of the family component[a]			
	Leaf area per seedling		Live leaves per seedling	
	P[b]	C:P	P	C:P
	%			
July 27	100**	0.0	100**	0.0
August 7	100**	0.0	100**	0.0
August 21	63.8	36.2	36.4	63.6
September 7	29.9	70.1*	0.0	100*
September 21	16.9	83.1**	0.0	100*
October 9	42.6	57.4*	23.0	77.0
October 24	0.0	100**	0.0	100**
November 9	0.0	100	0.0	100*

[a] * = Significant at $P \leq 0.05$ and ** = Significant at $P \leq 0.01$.

[b] P = Variance component among pairs, and C:P = Variance component between two reciprocal crosses within each pair. Negative estimates were given a value of zero.

Reciprocal effects (variation between reciprocal crosses within each pair) were found for number of live leaves and leaf area after August and increased toward the end of the growing season (Table 4). These effects were primarily related to the behavior of the two reciprocal crosses between G106 and L108 (Figure 4). Cross G106 x L108 demonstrated poor leaf

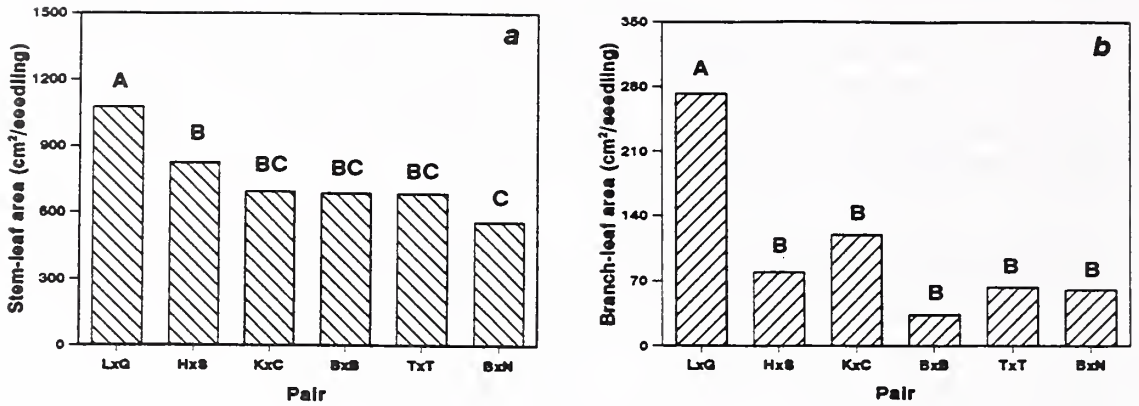

Figure 2. Mean comparisons of stem-leaf area (a) and branch-leaf area (b) in July for six pairs of sycamore seedlings (abbreviations of the pairs are given in the Materials and Methods). Bars not labeled by the same letters differ at $P \leq 0.05$ (experimentwise error at $P \leq 0.23$).

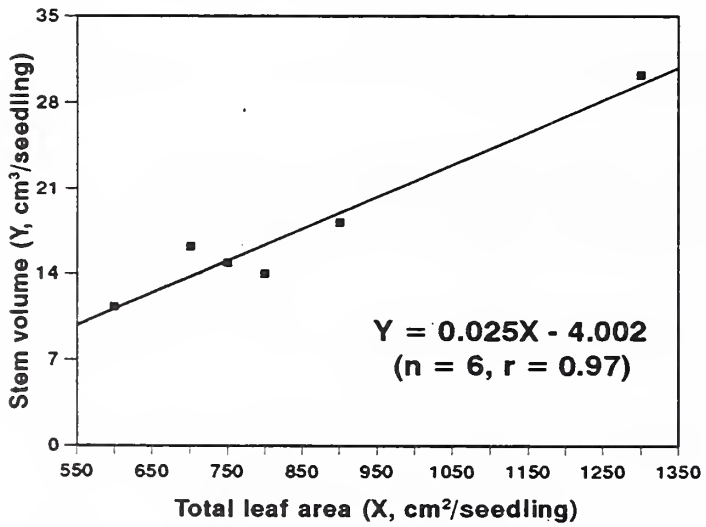

Figure 3. Relationship between leaf area per seedling in July and stem volume in December during the 1989 growing season (based on the means of six pairs of crosses).

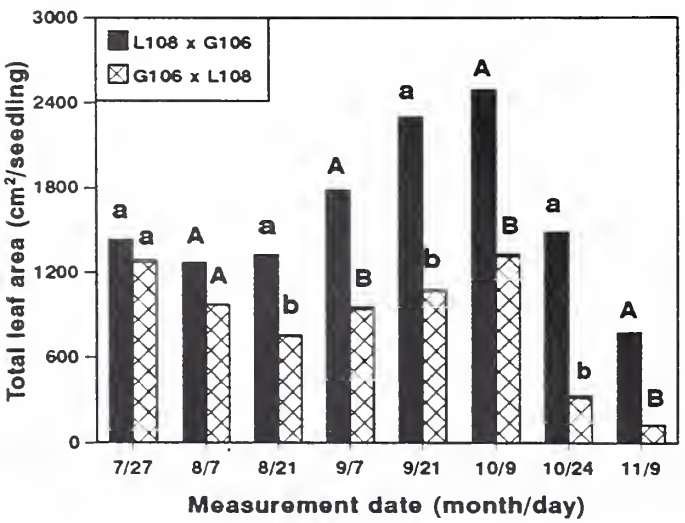

Figure 4. Mean comparison of total leaf area for two reciprocal crosses within pair L x G of sycamore seedlings from July 27 through November 9, 1989. Bars not labeled by the same letters at a given measurement date differ at $P \leq 0.05$.

retention during the August dry period, and it also had a slow recovery of leaf area during September to mid October after the dry period. However, cross L108 x G106 maintained nearly twice as much leaf area per seedling during the same time interval. The reason for this reciprocal effect cannot be explained by the present study, but as noted earlier it coincides with the decline of mother clone G106 from apparent graft incompatibility.

SUMMARY

Genetic differences were detected for (1) seedling height and diameter during most of the growing season, (2) total number of live leaves and leaf area per seedling before mid-August, and (3) stem volume at the end of the growing season. Reciprocal effects were responsible for family variation in seedling height one month after planting and then gradually disappeared. Differences among families for stem volume in October and leaf area production after August were also associated with the reciprocal effects. The fastest-growing cross produced the largest total leaf area per seedling early in the growing season, and it sustained this advantage with the greatest height, diameter, and stem volume growth throughout the growing season. Future research efforts need to focus on relationships between mother tree vigor, seed production and quality, and reciprocal effects on first-year seedling growth and leaf area development.

ACKNOWLEDGEMENT

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RESEARCH NEEDS AND THE VALUE OF FOREST BIOTECHNOLOGY AT UNION CAMP CORPORATION

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Abstract: -- Union Camp has had an active research program in forest biotechnology for twelve years. The first objective of this program is to develop genetically improved planting stock and deploy these as clones on an operational scale. Once superior clones are identified, the next objective is to make further gains in productivity through genetic engineering. Toward this goal, in 1995, Union Camp became the first US Forest Products Company to field test genetically engineered clones of sweetgum.

Our experience allows us to assign a relative value to forest biotechnology (i.e., genetic engineering and genetic mapping). In addition, it has brought sharply into focus the research needs for operational clonal forestry and the non-traditional genetic improvement of pine and hardwood. The research needs and the value of biotechnology from Union Camp's point of view will be presented.

Keywords: Biotechnology, clonal forestry, genetic engineering, genetic mapping

INTRODUCTION

The goal of Union Camp's forest biotechnology program is to improve forest productivity, (i.e., growth rate) by assisting in the development of clonal forestry in loblolly pine and hardwoods. Once superior clones have been identified, further gains in productivity are expected to be made through genetic engineering of those clones. Union Camp also has a program in genetic mapping. The objective of the mapping program is to characterize the individual genetic factors that contribute to growth rate. Loblolly pine is the focus of the mapping effort, not only because relevant mapping population are available in that species, but also because the development of molecular markers for growth rate would enable the use of cloning techniques developed for immature, but unproved genotypes.

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To successfully develop plantations consisting of genetically engineered clones of pine and hardwood, three components are necessary - germplasm, large-scale vegetative propagation and transformation. This paper will outline Union Camp Corporation's efforts in each of these three areas for both loblolly pine and a representative hardwood, sweetgum. Areas in which further research is needed to make operational plantations of genetically engineered trees a reality are highlighted.

HARDWOOD BIOTECHNOLOGY (SWEETGUM)

Species. Sweetgum has been the hardwood species that has received the most attention. Sweetgum is the "russet potato" of Southeastern hardwood species. That is, sweetgum is not outstanding in any one characteristic, but is good in many different traits. For example, sweetgum will grow on a wide variety of sites, has few disease and insect pests, and is an acceptable furnish for bleached Kraft pulps. From a biotechnological standpoint, it is relatively easy to vegetatively propagate (from tissue culture and rooted cuttings) and genetic transformation methods are well established.

Germplasm. Unlike loblolly pine, Union Camp does not have a long tradition of selection and breeding with sweetgum. Neither can we afford to wait 40 years until we have achieved the same level of success with hardwoods as we have with pine. Therefore our short-term to sweetgum improvement program is to select trees from genetic tests and test them as clones. The success of this approach is based on our ability to vegetatively propagate selection age trees. Cloning selection age trees has been a viable approach.

Shoot cultures from selection age trees are established by the method of Sutter and Barker (1985). Plantlets micropropagated from mature trees are then used as stock plants for rooted cutting production. Selected trees are tested as clones by propagating them as rooted cuttings from the micropropagated stock plants. Clonal testing has been underway for nine years and during this period, several hundred selections have been evaluated. At present, a small number of these clones have been chosen for scale-up. The magnitude of genetic gain in growth rate projected with these clones is similar to that seen in eucalyptus (Wright 1995).

Vegetative Propagation. Methodology for the large-scale production of sweetgum from rooted cuttings has been developed. Stock plant management, rooting conditions (both in containers and in the nursery), and regimes to grow rooted cuttings into plantable seedlings are in place. Micropropagation was used initially for clone capture (as noted above) but is now under investigation as a means of large-scale vegetative propagation.

Genetic gain in volume growth from utilizing clones (in the form of lower wood costs) will not be realized if the cost for producing clones planting stock is too high. Figure 1 shows the break-even cost of planting stock (relative to seedlings) as a function of genetic gain. A considerable increase in planting stock cost can be tolerated to vegetatively propagate elite material.

Micropropagation has traditionally been one of the most expensive ways to propagate woody plants. The labor associated with the intensive handling is the most significant component of the cost associated with micropropagation. However, the advent of automation technology may be able to go a long way toward reducing labor cost by eliminating the multiple handling of cultures and microshoots. Figure 2 gives an indication of how the propagation rate (i.e., the number of microshoots harvested from culture and set as cuttings) influences the cost of planting stock. Propagation rates on the order of four shoots per minute are needed to match the cost of seedlings. These propagation rates require that conventional, agar-based tissue culture systems become about 100 times more productive, and that each microshoot be touched only once by human hands.

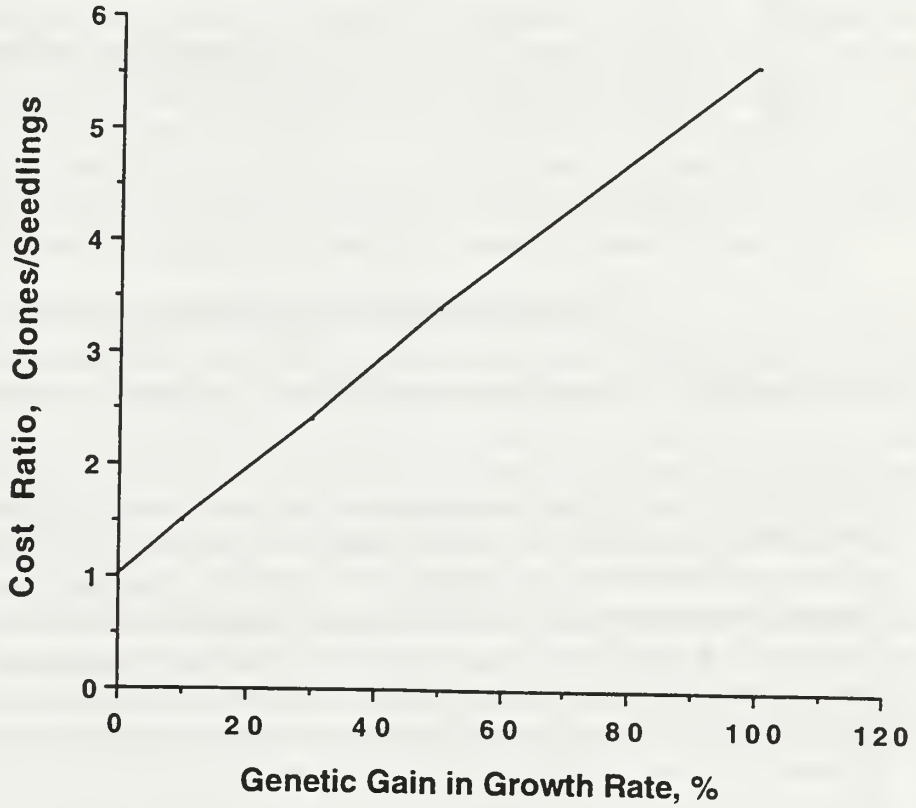

Figure 1. Break-even cost of sweetgum clones (relative to seedlings) as a function of genetic gain.

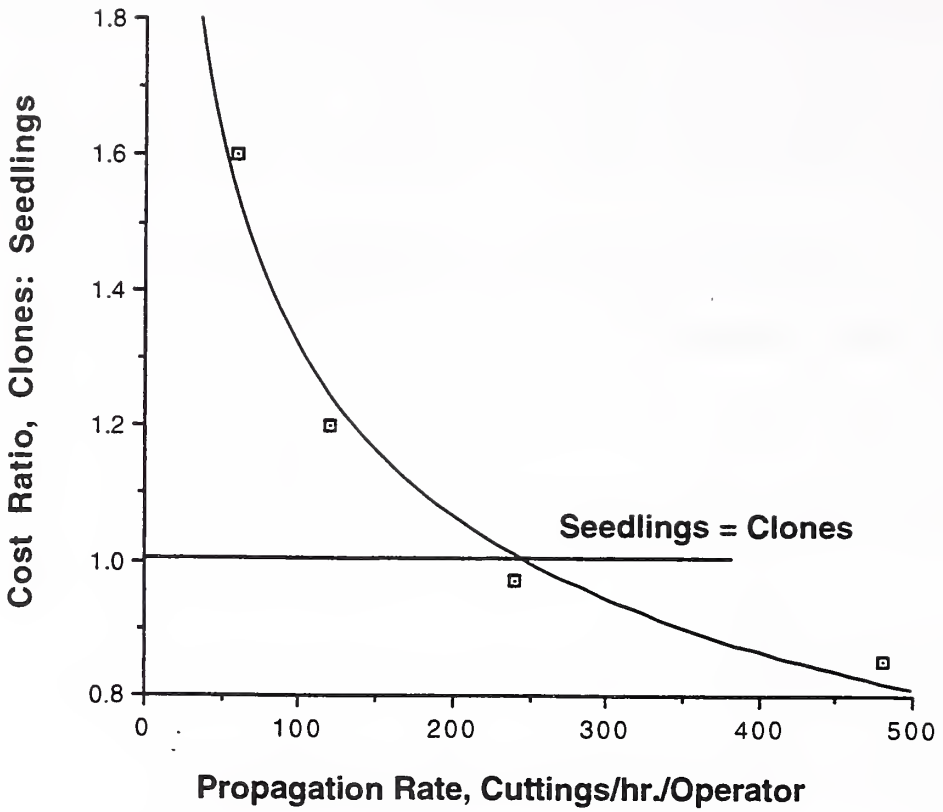

Figure 2. Effect of propagation rate on the cost of vegetatively propagated of planting stock.

Developing low cost vegetative propagation methods (for both pine and hardwood) is an area where there is a considerable need for innovation if we are to bring about clonal forestry in both pine and hardwood. Government and university researchers have avoided investigation of propagation systems as it is difficult to get funding for what is considered technology, not science. As a result, we find ourselves in the frustrating position of having genetically improved material that we cannot deploy as clones on a significant scale. To many tissue culture remains an art, and many laboratories continue to rely on procedures developed in the 1950s.

Genetic Transformation. *Agrobacterium*-based transformation of sweetgum has been previously reported (Feirer and Wann, 1989). This transformation method coupled with regeneration of shoots from leaf pieces (Brand and Lineberger, 1988) is routinely used at Union Camp to introduce foreign genes into elite clones of sweetgum. Our program has proceeded to the point that in 1995, Union Camp became the first US Forest Products Company to test genetically engineered trees in the field.

Union Camp's field test consists of sweetgum transformed with the *tfd*A gene, a gene that encodes for a enzymes that detoxifies the common herbicide 2,4-D (Stermer and Wilmitzer, 1989). In some cases, trees resistant to 2,4-D under greenhouse conditions did not express a

level of resistance in the field that would be suitable for operational use. This observation underscores the need to test each genetic transformation event individually.

Herbicide resistance would be of considerable value to hardwood plantation forestry. For example, if site preparation and vegetative competition control costs could be reduced to that of pine (as pine is basically herbicide resistant) the reduction in hardwood production cost could be as much as 25%. While herbicide resistance is likely to be one of the first traits commercialized in transgenic trees, the real impact of genetic engineering in forestry will come when genes that exert major effects on growth are identified. For example, if a gene was discovered that increased growth rate by a factor of two, this would cut wood production costs in half. Identification of genes that can exert a major influence on growth is the second area of research needed in forest biotechnology.

Our USDA-APHIS Permit to field test genetically engineered sweetgum stipulates that we must monitor the trees for flowering and prevent the release of transgenes (in the form of pollen or seed) should they flower. Containment is required because our field test, located near Belleville, GA, is in close proximity to native populations of sweetgum. The need to prohibit flowering in our field test implies that the USDA will require sterility in the operational deployment of transgenic trees. Therefore, it could said that before we pursue any other traits, sterility has to be the number one trait to target for genetic engineering.

The regulations surrounding release of genetically engineered organisms are still changing. It may be that for those engineered traits that would not constitute a plant pest risk (i.e., the trait would not increase the "weediness" of the native population) the requirement for sterility might be waived. Even if sterility were waived, corporations may not wish to risk the potential negative public perception of lack of containment of transgenes even if they were for traits deemed benign by governmental agencies. Therefore, the need for sterile trees may hinge on non-technical issues more than anything else.

Approaches to genetically engineering sterility have centered on the disruption of the developmental genes that orchestrate flowering (Strauss et al., 1994). There is a need to extend this technology to our Southeastern species. It should also be appreciated that even if genetic constructs that conferred sterility were available today, it would take years to evaluate their effectiveness in the absence of techniques for inducing precocious flowering. Early flowering (or perhaps more appropriately, accelerate maturation) is needed to compress the juvenile phase to a time period that would allow rapid assessment of sterility. These experiments are inherently difficult to perform because they require the evaluation of a negative finding (i.e., no flowers) under otherwise permissive conditions (i.e., physiological phase change).

Early flowering can therefore be considered to be enabling technology for transgenic trees. Floral stimulation techniques in juvenile loblolly pine are well established (Burris et al., 1991), such that seedlings can be induced to flower in 2-3 years. Less is known about floral stimulation in hardwoods. Techniques that will reliably induce flowering in hardwoods in as short a time frame as possible are therefore an urgent research need.

Germplasm. Union Camp has been selecting and breeding loblolly pine for more than forty years. This program has progressed from a time when planting stock was comprised of seed orchard mixes, then single, open pollinated family plantings and soon, full-sib families. Largely due to the foresight of Marvin Zoerb, large blocks of trees from full-sib crosses were planted as long ago as thirteen years in anticipation of a time when we would be planting full-sib families operationally. These planting have proved to be ideal mapping populations for the development of molecular markers (see below).

Vegetative propagation. Union Camp is actively investigating vegetative propagation of pine through rooted cuttings, micropropagation and somatic embryogenesis. Vegetative propagation from tissues older than seedlings has not been particularly fruitful, and attention has been focused on cloning options based on juvenile starting material - most notably somatic embryogenesis. Embryogenesis has good potential for scale-up as evidenced by the activity of several commercial concerns for pines and spruces. Embryogenesis is also appealing as a means of genetic transformation of conifers. This year Union Camp will establish its first clone test using somatic seedlings.

The use of juvenile starting material to develop clones would benefit greatly from early selection. Union Camp has been involved with physiological selection based on microcalorimetry since 1991 (Wann et al, 1991). Recently, we have extended our early selection program to genetic mapping. Molecular markers for growth would be of tremendous benefit since they have the potential to be independent of both age and environment.

Our genetic mapping program initially begun as a research contract utilizing our selection age full-sib plantings. Figure 3 shows a frequency distribution of volume index in one of these populations. Table 1 depicts the effect that marker-aided selection would have on wood cost if markers could be found that afforded the selection of trees with the percentage of volume above the mean shown. Marker selected trees would have to produce greater than 15% more wood above the mean to recover the costs of screening and vegetative propagation. However, if markers could be found that accounted for even 25% of the variation, their value to a mid-sized forest products company such as Union Camp would be well into millions of dollars per year. Markers have been found that account for a 7% increase in biomass (in the form of increased wood specific gravity) in radiata pine (Gleed et al., 1995) and 161% increase in volume in a hybrid eucalyptus cross (Chaparro et al, 1995). Given this spread in variation accounted for, it seems entirely reasonable that genetic markers that account for a significant portion of the variation in growth rate will be found in our mapping populations.

Table 1. Postulated effect of genetic markers for volume growth on pine wood costs (Base case = full-sib seed)

Wood Volume, % Above Mean	Wood Cost Ratio, Marker Selected/Full-sib mean
10	1.04
25	0.92
50	0.77
75	0.66

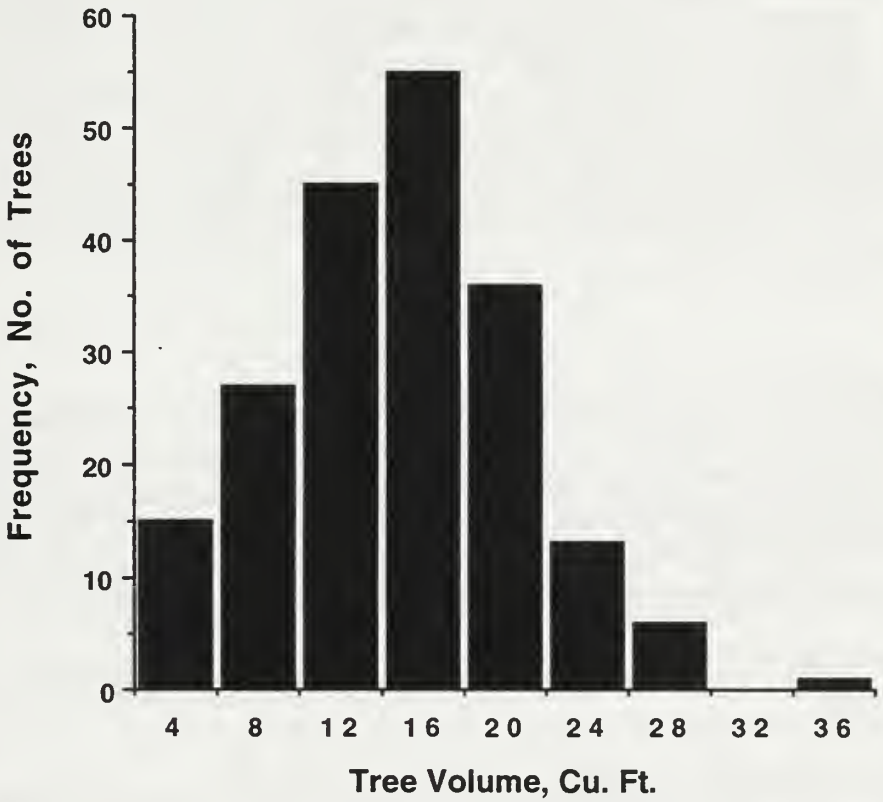

Figure 3. Volume distribution of a ten year old full-sib loblolly pine mapping population (location 1; n=200)

Large-scale plantings of full-sib crosses of selection age trees are ideal mapping populations and facilitate the search for molecular markers for growth rate. However, it would be extremely difficult to develop mapping populations for every parental combination we would want to use. For that reason, it would be highly desirable to have markers that are independent of genetic background. Markers that fall into this category are the genes

themselves, or simple sequence repeats (SSRs). Developing genetic markers that were independent of genetic background is a research priority.

Genetic Transformation. Unlike hardwoods, genetic transformation of loblolly pine is not yet routine in our laboratory. *Agrobacterium* has been used to transform embryogenic tissue of loblolly pine (Fierer and Wann, 1989). Further, transgenic conifers have been regenerated from embryogenic tissue (Ellis et al., 1993).

As with hardwoods, we have asked what traits should be targeted for genetic engineering. As noted, herbicide resistance is of limited value in pine. However, the Nantucket Pine Tip Moth causes considerable damage to young trees is a potential target for genetically engineered resistance. Noteworthy in this regard is the observation that several bt toxins are effective against tip moth. We postulate that a 10% reduction in wood costs could be made if engineered resistance to tip moth replaced spraying with pyrethrins. While insect resistance offers some reduction in wood costs, this reduction would pale in comparison to what might be accomplished if genes were found that had a major impact on growth rate. As with hardwoods, discovering single genes with profound effects on the growth of pine is an area where research is needed.

CONCLUSIONS

Throughout this paper we have highlighted where progress needs to be made before forest biotechnology can be utilized on an operational scale. The research topics identified are broad in scope, and as such are ideally suited for academic or governmental research. Value to forest products companies can only be derived from research in the above areas when an organization applies the findings to its own particular set of circumstances or genotypes. The areas where forest biotechnology research is needed are summarized below:

- Large-scale, cost-effective vegetative propagation technology for both pine and hardwood.
- Genetic constructs that, when transformed into trees: (1) confer sterility and (2) increase vegetative growth rate.
- Tissue-and age-specific promoters for transgenes.
- Early flowering in hardwoods.
- Molecular markers for growth, especially in loblolly pine, that are independent of genetic background.

Developing clonal forestry with genetically engineered trees is a daunting task and technical barriers still remain. While Union Camp has enjoyed a measure of technical success, the progress we have made has also been dependent on findings from academic and governmental

laboratories. By working together with the research community, we hope to help focus efforts on what should be done, rather than what can be done.

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Contributed Presentations: Extended Abstracts

PREDICTING GERMINATION CAPACITY OF SCOTS PINE AND NORWAY SPRUCE SEEDS USING TEMPERATURE DATA FROM WEATHER STATIONS

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INTRODUCTION

In Fennoscandia both Scots pine and Norway spruce often fail to produce mature seed, especially in the northern parts. In order to maintain sustainable multi purpose forestry cone and seed crop predictions are important. To determine if the seed crop is good enough for cone collection or natural regeneration, there is a need of early, large scale predictions of germination capacity. Identification of larger areas, regions, with good or bad conditions for seed maturation is important in an effective planning of both cone collection, soil treatment for natural regeneration, and to be able to concentrate the sampling on stand level to areas with the best conditions.

In Sweden the present method to predict germination capacity of Scots pine (*Pinus sylvestris* L.), on a large scale, is based on the temperature for the months June until August (Alfjorden and Remröd, 1975). The method needs improvement since the predictions may be inaccurate, especially for years with high temperature during early spring. Functions for Scots pine are used also for Norway spruce (*Picea abies* (L.) Karst.), even though Norway spruce may have lower temperature requirements for producing mature seed than Scots pine (Wennström and Almqvist, 1995).

MATERIAL AND METHODS

Cone samples used for obtaining seed germination data for this investigation were collected from natural stands throughout Sweden during 1971-1994. Data from 1297 Scots pine and 597 Norway spruce stands were used. The altitude of the stands varied between 5 and 700 m.a.sl. and the latitude varied from 55° 25' N and 68° 30' N for both species. All germination analyses were made immediately after the cones were collected and extracted. Number of seeds analysed was 300 (250-400). Seed samples with more than 50% seeds damaged by insects were excluded.

Daily mean temperature data from 71 stations of The Swedish Meteorological and Hydrological Institute (SMHI) were used. To each stand a temperature regime from the closest SMHI station was assigned. Temperature sums, d.d., with threshold values of +4° - +10°C were calculated from the start of the growing season until August 31 and

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September 30, respectively. Temperature sums needed for fertilisation to occur in each stand were estimated by first calculating the long time average temperature sum (threshold +5°C) for the stand (Morén and Perttu, 1994). Then the temperature sum needed for fertilisation was computed as a proportion of the long time average temperature sum. This proportion is about 30% for Scots pine and Norway spruce (Sarvas, 1967; Sarvas, 1968). Using the temperature needed for fertilisation as a threshold we calculated the number of days available for seed and embryo growth and maturation, NumD, as the number of days between the estimated time of fertilisation and August 31, which was used as estimate of embryo growth cessation.

Functions were created with the logistic regression model (Collett, 1991).

RESULTS AND DISCUSSION

Accumulated temperature sum (+5°C) from start of growing season until August 31 in combination with number of days from estimated time of fertilisation until approximate time for embryo growth cessation gave the best function. According to the developed functions the temperature sum requirements for producing mature seed of Scots pine were higher than for Norway spruce. Maximum difference in germination capacity was about 27 per cent units at 670 d.d. A germination capacity of 95% was reached at 875 d.d. for Norway spruce and at 975 d.d. for Scots pine (Figure 1). A difference of 10 days in the number of days between fertilisation and end of embryo growth (NumD) had a maximum effect of about 14 per cent units on germination capacity at 700 d.d. for Scots pine (Figure 2).

Of the temperature sums with different threshold temperatures that we tested +4°C, +5° and +6°C were almost equally good. The most common threshold value used in Swedish forestry is +5°C (Morén and Perttu 1994). Thus, it seems most appropriate to choose +5°C as threshold for the functions.

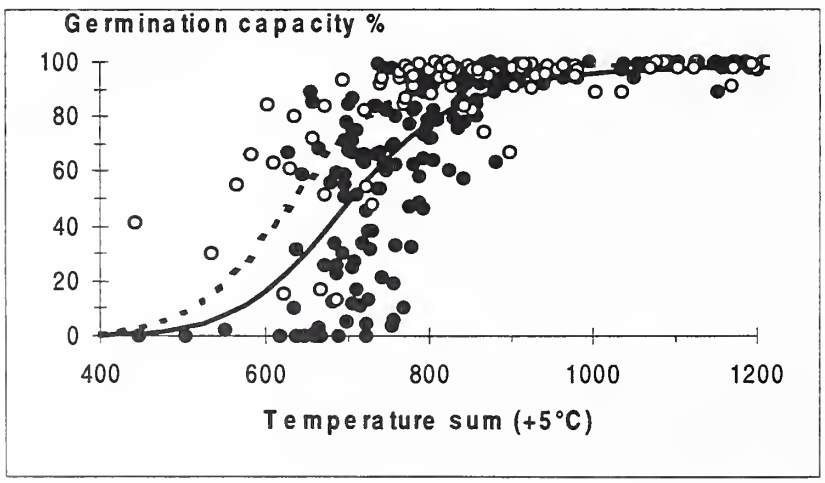

Figure 1. Relation between the germination capacity and temperature sum for Scots pine and Norway spruce seed based on functions with only temperature sum. Solid line and filled circles are Scots pine and dashed line and open circles are Norway spruce. A random sample of 200 Scots pine and 100 Norway spruce data points is included.

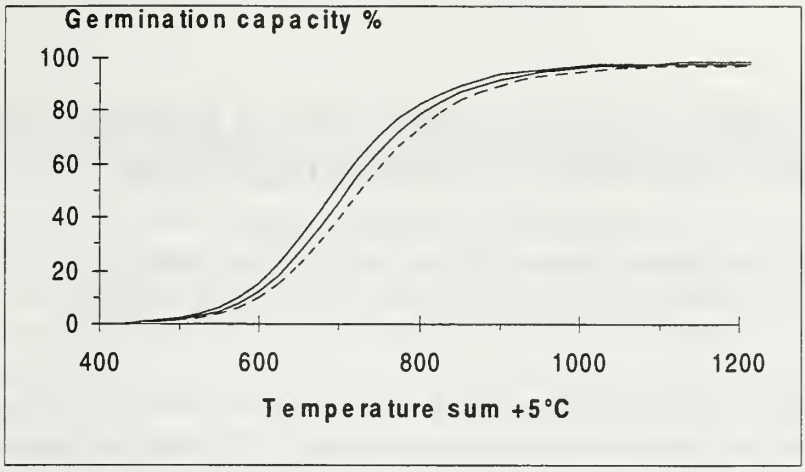

Figure 2. Response curves of the Scots pine function including the variable NumD (time from fertilisation to embryo growth cessation). Solid line is NumD=65, dashed line is NumD=70 and dotted line is NumD=75.

The time of fertilisation and consequently the time for the seed and embryo to grow and mature varies between years at the same locality. We used a rough estimate for this, in absence of more detailed information. A more correct way to calculate the time variable would probably improve the model.

CONCLUSIONS

It seems appropriate to use accumulated temperature sum (+5°C) from start of growing season until August 31 in combination with number of days from estimated fertilisation time until approximate time for embryo growth cessation as parameters in a function for predicting germination capacity of Scots pine and Norway spruce on a large scale.

Our functions show that Norway spruce has a lower temperature sum requirement for producing mature seed than Scots pine.

The presented functions could be used to identify regions with low or high risk of having weather conditions unfavourable for seed maturation.

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DELIVERY OF GENETIC GAIN: CLONAL ESTABLISHMENT AND DELIVERY VIA SOMATIC EMBRYOGENESIS

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Somatic embryogenesis (SE) is a tissue culture method of propagation which can be used for multiplying top-ranked families or capturing a greater proportion of the additive, dominant or epistatic genetic gain through the selection of elite clones (Mullin and Park 1992). The traits which could be captured via SE are those which can be identified through any conventional breeding program. These include yield and wood quality, and resistance to stress, pest or disease. Additionally, SE facilitates the introduction of value-added traits through genetic transformation (Ellis et al. 1993).

In forestry, SE fulfills a role similar to rooted cuttings for multiplication of families. The advantages include an achievable lower production cost (Grossnickle et al. 1997) and storage of germplasm via cryopreservation (Cyr et al. 1994). The latter enables the regeneration of valuable genotypes for operational production following selection from clonal field trials. Embryogenic lines can be initiated from embryos of mature (spruce) or developing seed (Douglas-fir, pine spp.). Technology development has focused on various spruce species (Roberts et al. 1991, 1993; Park et al. 1993), lobolly pine (Handley et al. 1994), Douglas-fir (Gupta et al. 1994) and radiata pine (Smith et al. 1994). Since 1988, the Forest Biotechnology Centre (BCRI) and Silvagen Inc. have been active in the development of SE as a delivery system of elite genetic material for reforestation.

Interior spruce (*Picea glauca/engelmannii* complex) is a major commercial species in British Columbia (BC), Canada. A tree-improvement program implemented by the BC Ministry of Forests (BCMoF) has provided the opportunity to apply SE to the capture of height-gain (Sutton et al. 1993), and to take advantage of family and potential clonal differences in weevil resistance (*Pissodes strobi*) (Kiss and Yanchuk 1991, Alfaro 1996). The breeding, testing and selection of parents for additive gene effects is the main emphasis of the program (Kiss 1968). First-generation clonal seed orchards have been rogued and genetic gains were about 11% based on 10-year height results (Kiss and Yeh 1988). Clonal testing of progeny from selected parents will capture additional gain over and above gains from rogued seed orchards.

The Prince George (PG) Selection Unit, accounting for 60% of the 100 million Sx seedlings planted annually in BC, is the source of material for the interior spruce (Sx) SE clonal selection program. This was initiated in 1990 and utilized 12 full-sib families selected from a base population of 174 trees (Dr. G. Kiss, BCMoF). These families were ranked low, medium and high for growth and weevil resistance. Approximately, 250 embryogenic lines were produced and stored in cryopreservation at BCRI. Somatic seedlings (emblings) representing 181 lines were deployed in field trials during 1994-95 (K. Thomas, Dr. C. Hawkins, BCMoF).

A second clonal selection program for Sx was launched in 1993. This was devised to accelerate the selection of high-yielding and weevil-resistant genotypes by utilizing the top 5 to 10% of the 1st generation parents from the PG Selection Unit (Sutton et al. 1993). In brief, the design aimed at the field testing of 1,000 clones from 30 to 40 full-sib families on 2 to 3 sites selected on the basis of high-growth potential or high weevil-hazard. Based on a 5% selection intensity, a total of 30 to 50 clones will be chosen for operational implementation. The selection of value-added genotypes will be initiated at 5 to 6 years after initial outplanting. Since the previous clonal trial was based on the same 1st generation population, it is expected that genotypes from those trials will augment the operational population; the first selections will commence in 1999.

Approximately 1,900 Sx embryogenic lines representing 48 ranked families have been stored in cryopreservation since 1992. This clone bank has facilitated the delivery of 571 lines (21 full-sib families) to clonal field trials during 1996-97 (Dr. C. Hawkins, BCMoF). To increase genetic diversity, a new population of open-pollinated (OP) weevil-resistant families was added to the program (Quesnel Lakes). Fifteen top-ranked parents were selected from a base population of 140 trees based on progeny field tests for weevil resistance (Dr. R. Alfaro, Natural Resources Canada [NRCan] and Dr. G. Kiss, BCMoF). Based on current nursery inventories, an installment of up to 700 lines from 15 families for the 1988 clonal field trials is expected. This will ensure a delivery of up to 1,200 lines from 48 families for the 1996-98 clonal selection program. Additionally, a subset of the families and lines represented in the 1996 trials were installed in weevil screening trials by Dr. Rene Alfaro (NRCan).

Sitka spruce (Ss: *Picea sitchensis*) is a valuable coastal species which has been decimated by the pine weevil. The annual planting has declined by more than 90% from a historical high of 10 million seedlings per annum. Consequently, less-desirable species such as western hemlock and western red cedar are being planted as replacements. In response to these pressures on coastal reforestation, a breeding program for weevil-resistant Sitka spruce has been initiated during the past decade (King 1994).

A SE program for weevil-resistant Ss started at BCRI in 1994, has resulted in a clone bank of 322 lines representing 22 weevil-resistant families, primarily open-pollinated. Emphasis is being placed on the highest ranked of 75 tested OP families (Jordan River Field Trial, Dr. J. King, BCMoF; Dr. R. Alfaro, NRCan). Clonal trials of 42 lines (11 families) were initiated in 1997 in collaboration with, the BCMoF (C. Cartwright, Dr. J. King), forest industry partners (Canadian Forest Products, International Forest Products, Macmillan-Bloedel, Western Forest Products, Weyerhaueser) and the Oregon Department of Forestry. Additionally, collaborations have been established for weevil-screening (Dr. R. Alfaro, NRCan) and cuttings protocol development (D. Summers, BCMoF). This Ss program is expected to accelerate, with approximately 175 lines targeted for deployment in 1998 clonal trials. The induction of new embryogenic lines from the top-ranked families is in progress (1999 clonal trials). This will facilitate increasing the clone bank to 500 lines.

Efforts at BCRI are now being focussed on SE programs for other conifer species. For Douglas-fir (*Pseudotsuga menziesii*), 250 embryogenic lines from 10 high-yield families have been stored in cryopreservation. This complement is expected to increase by 100 lines during

the summer of 1997. Additionally, embryogenic lines have been established for several pine species with 170 of 400 of the lines currently stored in the clone bank.

Sx and Ss have been scaled to commercial production by Silvagen Inc. during the past 18 months with over 750,000 SE propagules produced to date. In 1997, 200,000 Sx somatic seedlings (spring and summer-ship) are being delivered to operational trials, while 180,000 Sx (12 full-sib families) and 15,000 Ss (12 lines) currently in nursery production for deployment in 1998. Pilot-scale production has demonstrated a current capacity of over 1 million somatic embryos annually. Deployment guidelines (under development by the BCMoF), currently require an effective population of 10 and 5 lines per full-sib family; the SE production for 1998 stocks will satisfy these requirements.

Keywords: *Picea glauca engelmannii, Picea sitchensis, Pseudotsuga menziesii,* pine, somatic embryogenesis, selection, clone bank, genetic gain.

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MICROSATELLITE MARKERS FOR LOBLOLLY PINE

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We are developing PCR-based codominant microsatellite markers for loblolly pine. Until now there has been a shortage of fully informative PCR-based codominant markers for loblolly pine. In outbred pedigrees, heterogeneity of mating types at each marker increases the number of segregating progeny required for mapping. Multi-allelic markers are more likely to be fully informative, and reduce the number of progeny required in outbred pedigrees. Microsatellite, or simple sequence repeat (SSR), markers produce the highest proportion of fully informative genetic markers, which are especially advantageous to QTL detection and population studies in outcrossing species. The multi-allelic nature of SSR markers can reduce bias in detecting quantitative trait loci for loblolly pine by increasing the proportion of heterozygous parents. Furthermore, since SSR markers require little DNA and no radioactivity, they can be automated for high-throughput data analysis.

Microsatellite sequences were recovered from our first loblolly pine genomic plasmid library enriched for $(CCT)_n$, $(GGT)_n$, $(CGT)_n$ and $(GCT)_n$. Library screening revealed that 32% of the clones were positive for trinucleotide repeats. Of the positive clones, 236 have been sequenced and all have been found to contain trinucleotide repeats, up to 70 repeat units in length. Of the clones sequenced, 62 are unique, indicating that our enrichment technique resulted in 74% duplication of sequences. Of the 74% duplication, 65% resulted from two sequences, most likely a result of PCR bias. This problem is now being alleviated by decreasing the number of PCR amplification steps and number of cycles per PCR step in the enrichment procedure. Of the unique clones with sequences flanking the microsatellites sufficient for primer design, 16% contained large repeated motifs in regions proximal to the microsatellites, rendering it impossible to design unique primers. This may be a unique characteristic of the pine genome.

BLAST (Basic Local Alignment Search Tool) results suggest that a high proportion of these microsatellites are proximal to coding regions. The BLAST algorithm measures local sequence similarity based on a matrix of similarity scores for all possible pairs (Karlin and Altschul 1990; Altschul et al. 1990), and detects aligned pairs of sequence segments which are "high scoring segment pairs" (HSPs). Probability of a random match is estimated using the Poisson probability, with a probability, P(N), of 0.05 as the cutoff value for significant HSPs. Our 62 unique sequences were compared to sequences in nucleotide and amino acid sequence databases using BLAST. Microsatellites and other repetitive regions in the query sequences were eliminated by low-complexity filters. BLASTN, which searched for significant HSPs between the query nucleotide sequence and nucleotide sequences in the Genbank, EMBL, DDBJ, and PDB nucleotide databases, indicated similarity to eukaryotic sequences for 21 of the microsatellite clones. BLASTX was used to find significant HSPs between translated nucleotide query sequences in all six reading frames and target amino acid sequences in Genbank, CDS, PDB,

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Swisprot and PIR protein databases, revealing significant HSPs between 13 of the microsatellite clones and eukaryotic proteins. Four of the clones matched sequences using both BLASTN and BLASTX. Therefore, 48% of the clones matched nucleotide and/or amino acid sequences of eukaryotes in the databases. Among the matches in BLASTN is a sequence that is similar to a *P. taeda* promoter for an arabinogalactan-like protein gene with $P(N) = 4.3 \times 10^{-17}$. BLASTX revealed two sequences that are similar to retrotransposons with $P(N) = 5.7 \times 10^{-28}$ and 8.8×10^{-23}, and sequences similar to *Nicotiana tabacum* extensin and *Zea mays* CRINKLY 4 precursor, with P(N) = 0.00016 and 0.011, respectively.

Microsatellite markers that are in close proximity to coding regions are especially valuable. Our working hypothesis is that many SSRs are directly involved in gene expression and function. For example, variation in repeat number may cause quantitative changes in gene expression (Kashi et al. 1997). Potential applications of this type of marker are chromosome landing, QTL mapping, and molecular systematics.

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PROGRESS TOWARD OPERATIONAL DEPLOYMENT OF LOBLOLLY AND SLASH PINE ROOTED CUTTINGS

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The use of rooted stem cuttings of loblolly and slash pines offers an opportunity to capture additional genetic gain in plantations. Using rooted cuttings with high genetic performance values as planting stock is becoming increasingly common with many species around the world. Rooted cuttings are being utilized to capture genetic gain at two levels. The first multiplies seed from previously tested, outstanding crosses. This approach delivers genetic gain equivalent to mass controlled pollination and could be important when seed is in short supply, such as when superior crosses are first identified, but before they can be established in seed orchards and grown to sufficient size to meet reforestation objectives. The ultimate utility of both full-sib deployment methods will depend on the cost effectiveness and amount of genetic gain delivered in a given period of time. The second level of genetic gain to be exploited is that which comes from propagation of individual clones. This will deliver the most genetic gain, provided an efficient system can be developed for within-family selection and propagation.

Successful operational deployment of rooted cuttings will depend on efficient production procedures and acceptable field performance of rooted cuttings. One critical element of efficient production is the reliable rooting of many genotypes at high percentages. Significant improvements in rooting percentages have been achieved by implementing recent research results. In one study testing the effects of rooting powder treatments on cuttings from 4-year-old stock plants (hedges) of four full-sib loblolly pine families, the rooting percentage of all four families was 86% across all treatments (excluding controls). In another study screening cuttings from 2-year-old hedges from 25 open-pollinated loblolly pine families, the overall rooting percentage was 74% and 72% of the families rooted at 70% or higher.

One of the most important factors in achieving good rooting success is the production of large numbers of uniform cuttings with juvenile morphology and growth habit through rigorous hedge management. Research on hedge fertilization has revealed a beneficial effect of maintaining nitrogen tissue concentrations well above those normally found in bare-root seedling crops. Another experiment indicates that deficiencies of the micronutrient boron can effect rooting success. Cuttings from four full-sib loblolly pine families rooted at 67% when the concentration of boron in the tissue was less than 10 ppm, but rooted at 81 to 87% when the tissue boron concentration ranged from 11 to 35 ppm.

The rooting environment is being improved using mist application systems that have a high degree of uniformity and by research on the physiological processes in cuttings and hedges. Recent results have identified critical levels of water stress beyond which rooting performance declines and handling and storage practices that maintain the water status of cuttings above these

critical levels. Experiments are also underway to test the effects of light, temperature and CO_2 concentration on rooting.

Additional increases in rooting percentages can be achieved by culling poor-rooting families or individuals. Culling poor rooting families should not impact genetic gain for growth rate or rust resistance, as rooting ability is independent of these traits in open-pollinated slash pine families. Tests of correlations between rooting and growth are also currently underway for loblolly pine families and early results suggest independence.

Improvements in the morphology of cutting root systems, but not necessarily rooting percentage, have been achieved through the use of specific hormone treatments. Application of the synthetic auxin, NAA (1-naphthaleneacetic acid), increases the number of roots per cutting and the percent of cuttings with symmetrical root systems in dormant (winter) loblolly pine cuttings. NAA effects are less pronounced in succulent (spring and summer) loblolly pine and in slash pine cuttings. Results from a field test examining the effects of root morphology on growth of loblolly pine rooted cuttings reveal no significant effects of root number or root system symmetry on growth at the end of the first field growing season. However, growth, wind firmness and drought resistance will be monitored over the longer term.

Rooted cuttings must grow as rapidly as seedlings of equivalent genotype for this method of producing planting stock to successfully capture genetic gains. In addition, it is important to ascertain if family performance for rooted cuttings can be predicted based on seedling performance data. Six-year field results from loblolly pine seedling/cutting tests established in two locations indicate no overall differences between rooted cuttings and seedlings of the same full-sib families in height, diameter or volume. Rooted cuttings were consistently, though not significantly, less susceptible to fusiform rust infection. In addition, family mean correlations between seedlings and cuttings for the 9 full-sib crosses (3 x 3 factorial) were $r = 0.97$ for height growth and $r = 0.84$ for rust infection on the more uniform test site. Performance of the rooted cutting families was also highly correlated with independent progeny test data, indicating that full-sib families of rooted cuttings can be deployed without additional testing.

In order to implement true clonal forestry, maturation must be addressed. Juvenility must be maintained long enough to test and multiply individual clones before rooting percentages or the growth rate of rooted cuttings from hedges of these clones decline. A study to test the effect of hedging and serial propagation on the maintenance of juvenility in loblolly pine is underway. While definitive results from this study are not yet available, the maintenance of hedges with no apparent decline in rooting ability through four years (described above) is encouraging. A field test to determine growth rates and characteristics of the rooted cuttings from different aged hedges will be established this winter.

Results presented here indicate that full-sib multiplication of loblolly and slash pine is a viable biological procedure. Operational use will depend on the cost of producing planting stock relative to the additional genetic gain delivered. Clonal forestry with these species will depend on the success of procedures to maintain juvenility and, though not yet definitive, the combination of hedging and serial propagation appears to hold promise.

A GENOTYPE-INDEPENDENT METHOD FOR TRANSFORMATION OF PINES USING *AGROBACTERIUM*

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Forest products constitute the third most important cash crop in Texas after cotton and grain sorghum. Of the forest species, loblolly pine is the most important. The single most important factor blocking improvement of loblolly and other commercial pine genotypes through genetic engineering has been an inability to regenerate plants following transformation. To overcome this block in plant regeneration, we modified a direct shoot apex inoculation procedure using *Agrobacterium*, developed earlier for cotton and maize (Gould et al., 1991). Inoculation of a pre-formed shoot facilitates plant regeneration and makes the procedure genotype-independent. The potential for somaclonal variation caused by tissue de-differentiation in culture is reduced and total time in culture is minimized. The method exploits super-virulent *A. tumefaciens* (Hood et al., 1986;1990), transformation competent cells in the apical meristem and the native capacity of the shoot apex to regenerate into a complete plant. Using this method, we have recovered transgenic plants of loblolly pine and putative transgenic plants of afghan, radiata and Virginia pines. The evidence obtained for the genetic transformation of loblolly pine are: promoter-dependent GUS expression, PCR amplification of sequences unique to *nptII* in the DNA of regenerated plants, and high molecular weight DNA with homology to the transferred *uidA* (GUS) and *nptII* (kanamycin resistance) genes, characteristic of genomic incorporation.

Pinus taeda and other forest trees have only recently begun to be domesticated through selection and breeding. Significant improvement can be achieved through marker-aided selection and propagation of superior types. Specific and dramatic genetic gain may be possible through transformation with engineered genes; however, this achievement may be gained at the cost of genetic diversity since most plant transformation and regeneration procedures available are limited to regenerable genotypes. Although loblolly pine readily sustains genetic transformation by *A. tumefaciens* (Sederoff et al. 1986; Huang & Tauer, 1994) and particle acceleration methods, recovery of transformed plants from transformed tissue has been rare. To overcome these problems in plant regeneration from callus or embryogenesis following transformation, we used *A. tumefaciens* inoculation of the shoot apex (modified from Gould et al., 1991), and direct regeneration of plants from shoots. This approach exploits the evolved gene transfer mechanism of *Agrobacterium*, the dividing cell population in the apex that accepts *Agrobacterium* transformations, and the unique developmental characteristics of an apical meristem. The transformation method is genotype-independent and compatible with all pine species and existing tree improvement programs.

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Shoots from organogenesis in tissue culture or from germinating seedlings can be used in transformation, which permits application to elite and unique genotypes, seedlings identified through marker-aided selection, and can be adapted to clonal forestry.

Shoots of *Pinus taeda L.*, derived from cotyledonary explants were initiated as described (Jang & Tainter, 1991), separated and placed in shoot elongation medium (Meha et al., 1978; Mott & Amerson, 1981; Chang et al., 1991) until 0.5 to 1.0 cm in height. Shoot cultures were then stored at 4C for up to 3 months and then recultured on media containing a cytokinin 1 week prior to inoculation. Shoots isolated from germinated seedlings were also used. Shoots were inoculated with *A. tumefaciens* EHA101 (pGUS3) or EHA105(pSSLa). Both vectors carried *uid*A (GUS, beta-glucuronidase) and *nptII* (neomycin phospho-transferase II), the only difference being the promoter used with the GUS gene. The pGUS3 plasmid (Gould et al., 1991) contained the CaMV 35S promoter fused with GUS and was used in EHA101 (Hood et al., 1986: 1990), while pSSla.3 (Campbell et al., 1994), contained the larch Rubisco small subunit promoter fused with GUS and was used in EHA105. A single 4 week passage on a 50-75% lethal dose of 25 mg/l kanamycin was used. This value was chosen because it allowed escapes. The level of selection was intentionally kept low to insure survival of transformed shoots, since dividing cells in the shoot meristem are more sensitive to kanamycin than other cells. Approximately 10-20% of the inoculated shoots survived this treatment, although shoot mortality was not evident immediately. All shoots were recultured, and surviving shoots recovered and elongated to a 2 cm overall length in approximately 8-10 weeks. At this time, approximately 10% of inoculated shoots were ready for root induction; however, overall recovery of plants was low (1-3%) because of low rooting response. With development of a more efficient method for rooting loblolly shoots, recovery of plants can be greatly improved.

Elongating shoots and intact plants were assayed for GUS activity. Expression of GUS was promoter dependent: localized to phloem tissue with the CaMV 35S promoter (in pGUS3); restricted to emerging leaves flanking the shoot meristem with the Larch Rbcs promoter (in pSSLa). Southern DNA analyses showed transfer of both *uidA* (GUS) and *nptII* (kanamycin resistance) genes into high molecular weight DNA of recovered plants. PCR and Southern DNA analyses ruled out the possibility that the transferred genes were present in residual *Agrobacterium* contamination. We used the same transformation procedure with afghan, radiata and Virginia pines, and have recovered approximately 100 plants of afghan pine. These three species tolerate shoot meristem transformation well, root readily and can be useful in generating large numbers of transgenic plants for testing effectiveness of many genes and promoter sequences intended for use in loblolly or other pines.

In summary, we have used an *Agrobacterium* and shoot apex transformation method with loblolly pine and have recovered transgenic plants. Genetic fidelity is most closely maintained in the meristems of plants, and plant regeneration from isolated shoots and cuttings is straight-forward and simple. Isolated shoots are inoculated with a hyper-virulent strain of *Agrobacterium tumefaciens*, subjected to selection and generated directly into plants which makes the process genotype-independent. Tissues do not pass through a dedifferentiation

step to callus, and plant regeneration is not dependent on shoot organogenesis, somatic embryogenesis or limited to regenerable genotypes.

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DECISION-MAKING IN PROGENY TEST LOCATION USING GIBBS SAMPLING

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Abstract : The manner in which progeny tests are deployed affects genetic gain in production populations in the presence of genotype x environment interactions (GE). The conventional method of determining the site to locate progeny tests is to estimate the efficiency of selecting at one site for planting at another site. Since early selection is normally practised in tree breeding, efficiency of early selection across sites is important. In order to estimate the efficiency of selection, heritability and genetic correlations are estimated using standard methods such as REML. A major limitation of this approach is that the variance of the efficiencies of selection are unknown, adversely influencing efficiency of decision-making.

An alternative method is to use a Bayesian approach such as Gibbs sampling. This approach, is attractive because the sampling distribution of the efficiency of selection can be obtained and expected efficiency and variance of the efficiency of selection can be derived and futhermore, the probability that the efficiency of selection lies between certain values can also be estimated, thereby producing considerably more information on which to base decisions on compared to the point estimates from REML.

Data for the Gibbs sampler were heights assessed at ages 9.5 years and 22.5 years at two sites in Zimbabwe. The analysis was done using Multi Trait Gibbs Sampling for Animal Models program (MTGSAM, Van Tassell and Van Vleck 1995). The four traits were analysed simultaneously and a total of 1000 samples of genetic and phenotypic covariances were stored. From these, heritability, genetic correlations and efficiencies of selection were calculated for each sample, and inferences about efficiencies of selection were made by computing directly summary statistics from the distribution derived from the 1000 samples. These efficiency estimates were compared to the point estimates from REML. The efficiency of lower than 0.70 was assumed to justify extra costs of establishing separate progeny tests.

The estimated selection efficiencies are shown in Table 1. While the efficiencies of early selection across site showed little variation, those for selection at maturity across sites did. The probability that the efficiency of early selection at site C for planting at site A was greater than 0.70 was 0.93, indicating that early selection at site C would result in little loss in gain at site A at harvest age, compared to early selection at site A. In fact, the probability that more gain would be obtained from early selection at site C compared to site A is 0.2.

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Table 1. REML and Gibbs sampling estimates of efficiencies of selection for height and their standard deviations (SD), and the probabilities that the selection efficiencies (E) are greater than 0.7 and 1.0.

	REML estimate	Posterior mode	Posterior mean	SD	P(E>0.7)	P(E>1.0)
E_{A2c4}*	0.84	0.80	0.70	0.18	0.57	0.01
E_{c2A4}	0.75	0.75	0.89	0.15	0.93	0.20
E_{A4C4}	0.49	0.50	0.46	0.22	0.12	0.00
E_{C4A4}	0.53	0.63	0.48	0.23	0.17	0.01

*E_{A2C4} = efficiency of selecting at site A at 9.5 years for planting at site C compared with early selection at site C at 9.5 years.

E_{c2A4} = efficiency of selecting at site C at 9.5 years for planting at site A compared with early selection at site A at 9.5 years.

E_{A4C4} = efficiency of selecting at site A at 22.5 years for planting at site C compared to direct selection at site C.

E_{C4A4} = efficiency of selecting at site C at 22.5 years for planting at site A compared to direct selection at site A.

The efficiency of early selection at site A for planting at site C was greater than 0.70 was only 0.57, and only 0.01 that early selections at site A would result in higher gain at site C at harvest age than early selections at site C.

The results suggest that site C is a better progeny test site since selections made here will result in little loss in gain at site A, and may even result in higher gain at site A at harvest age whereas early selection early at site A would severely reduce progress at site C. If selections are to be made at maturity, results indicate that separate progeny tests should be established for the sites since selections at alternative sites would result in substantial losses in gain at the sites.

The decision regarding selection at maturity is consistent with that obtained using point estimates from REML, but Gibbs sampling allowed the efficiencies of selection to be interpreted with more confidence.

The decision regarding early selection differed from that based on point estimates from REML. Using REML, the efficiencies of early selection at both site A and site C were greater than 0.7 indicating that any of the two sites could be a suitable location for progeny tests, while with Gibbs sampling it was clear that site C was a better site to locate progeny tests. The results indicate that, even in this simply decision problem, Gibbs sampling can be an attractive approach to decision-making as more information to make inferences about the parameter of interest can be derived from the analyses than possible from REML. The benefits might be expected to be even greater in more complex decision processes.

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GENERAL PREDICTION MODEL FOR AGE-AGE GENETIC CORRELATIONS IN *PINUS TAEDA*

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Abstract:--In long rotation crops such as forest trees, traits are rarely measured at all ages up to harvest age. Therefore, modelling of age-age genetic correlations is essential for predicting optimum selection age. Using data from four progeny tests of Pinus taeda planted in Zimbabwe assessed up to rotation age for height at 1.5, 9.5, 13.5, and 22.5 years, a tropical prediction model for age-age genetic correlations was derived (Gwaze et al. 1996, $r_g = 0.98 + 0.065 \log_e$ (younger age/older age)). This model was significantly different from phenotypic models such as Lambeth's (Lambeth 1980). The tropical model was tested using data from twenty genetic tests of P. taeda from south eastern USA, assessed at 3, 5, 8, 10, 15, 17, 20 and 25 years (Table 1). The tests were located in Arkansas, Georgia, Louisiana and Texas. Possibilities of developing a general prediction model for P. taeda based on age-age genetic correlations were explored.

Table 1. Details of P. taeda genetic tests used in analyses.

Set	Organization	Sites	Parents	Reps/site	Trees	Assessments ages
1	International Paper Company	2	222	3	4251	3, 5, 10, 15, 17, 25
2a	Texas Forest Service	12	129	3-16	9647	5, 10, 15, 20
2b	Georgia-Pacific	5	61	3-9	14033	10, 15, 20, 25
2c	Crown Zellerbach	1	11	4	1475	3, 5, 8, 10, 15

Age-age genetic correlations were estimated using individual tree model ASREML (Gilmour et al. 1997). Genetic correlations were higher than family mean correlations. Strong linear relationships between age-age genetic correlations and log of age ratios were obtained (Table 2). The slopes of the regression models of the USA datasets were significantly higher than that of the tropical model. The main reason for the differences between these models is that genetic correlations involving very young ages were much higher in Zimbabwe tests than in the USA. The slopes of the western and eastern datasets were not significantly different. Thus, a pooled regression for all USA datasets was

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appropriate. The USA model for combined datasets was not significantly different from the Lambeth model despite that the former was based on phenotypic correlations.

Table 2. Parameter estimates for fitted models and fit statistics.

Model	β_0 (se)	β_1 (se)	RMS	df	R^2
Tropical	0.98 (0.044)	0.065 (0.026)	0.0032	4	0.61
Set 1	1.03 (0.015)	0.294 (0.013)	0.0009	13	0.97
Set 2a	1.02 (0.044)	0.204 (0.052)	0.0023	4	0.80
Set 2b	1.04 (0.022)	0.218 (0.039)	0.0005	4	0.89
Set 2c	0.97 (0.047)	0.253 (0.053)	0.0047	8	0.74
USA combined	1.04 (0.019)	0.284 (0.020)	0.0035	35	0.85

The study suggests that a general predictive model for both the tropical and temperate regions may not be appropriate. Therefore, two separate prediction models for P. taeda in the tropical and temperate areas should be derived, with the one derived here using USA data being appropriate for temperate areas. However, with high gentic quality seed and better site management in the advanced generation tests, genetic control of height at very young ages might be higher, and genetic correlations between very young ages and mature ages might increase. Under this hypothesis, the general model involving advanced generation USA tests and the tropical model might agree more closely.

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CLONAL-ROW AND RANDOM SEED ORCHARD DESIGNS: COMPARISON OF MATING PATTERN AND SEED YIELD

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Abstract: Research on first generation seed orchards' genetics revealed that most if not all were efficient in seed production, however, they often did not fulfil the assumptions required for attaining maximum genetic efficiency (El-Kassaby 1989). The replacement of first-generation seed orchards by second- and/or advanced generations is expected to be associated with appreciable increase in the genetic worth of seed crops. This replacement requires consideration of alternative seed orchard designs that allow for maximizing the genetic worth of future seed crops as well as ease of management. Clonal-row seed orchard design has been advocated by Greenwood (1983) as an alternative that should be given serious consideration. Evaluation of this seed orchard design was only restricted to seed yield (Bramlett and Bridgwater 1987). Genetic evaluation of this type of seed orchard design is imperative due to the non-random of placement of clonal ramets in the orchard grid.

This paper evaluates and compares the mating pattern (selfing rate and level of correlated matings) and seed yield between clonal-row and the traditional random design in a western hemlock (Tsuga heterophylla (Raf.) Sarg.) seed orchard. A western hemlock (Hw) seed orchard was selected for this study for the following reasons: 1) the species has: a- wide reproductive phenology differences among its clones (i.e., the chance of selfing and correlated mating increase), b- unique pollination biology that is characterized by the presence of true pollen competition (i.e., increased chance of success of unrelated pollen) and long receptivity period (Colangeli and Owens 1989, 1990), 2) tree height is kept at a maximum of 3m, thus facilitating pollen management, 3) all Hw orchards rely on supplemental-mass-pollination (SMP) as a standard pollen management practice, and 4) pollen contamination is extremely low, thus seed yield and mating pattern results do not suffer from confounding factors.

This study included: 1) reproductive phenology survey to determine the extent of the pollination season, 2) controlled selfing trial to determine clonal propensity to selfing, 3) comparison of seed yield, and 4) determination of selfing and correlated selfing rate using allozyme markers.

The results are summarized as follows:

1) Reproductive phenology : reproductive phenology survey of the entire orchard (i.e., 100% sampling) indicated that the pollination season is typical to that of other conifers studied (El-Kassaby 1992). It is characterized by the presence of early, medium, and late reproductively active clones and covered a period of 22 days. This extended pollination season is expected to produce a continuum of temporally isolated sub-breeding populations over time, thus increasing the chance of inbreeding and restricting the number of available

mates at any specific time. This could be further accentuated by the short duration of maximum pollen receptivity and shedding (Colangeli and Owens 1988).

2) Selfing trial: a total of 25 clones were self-pollinated and produced an overall selfing rate, as determined by the percent of filled seed, of 3.4% (SD= 6.16%). However, it should be emphasized that few clones showed higher propensity for selfing with percent of filled seed ranging from 8 to 29% . Controlled selfing, as an exploratory procedure for this seed orchard design, is recommended for identifying clones with high selfing rate. These clones should be targeted for SMP application.

3) Seed yield: seed yield from a random sample of 45 seed-cones from each of 66 (clonal-row) and 56 (random) trees was determined. The number of filled seeds per cone was determined (# of filled seeds/# of seed-cones sampled). Seed-cone size within both orchard designs showed large differences, thus seed-cone size was included in the analysis as a covariate. Seed yield analysis produced no significant difference between the two seed orchard designs. Thus, seed orchard design has no effect on seed yield.

4) mating pattern: mating pattern was determined from a sample of approximately 750 seeds representing 20 clones per orchard design. Outcrossing rate estimates were 0.899 and 0.970 for the clonal-row and random seed orchards, respectively. These estimates are significant from complete outcrossing (i.e., $t = 1.0$) and are significantly different between the clonal-row and random seed orchard, indicating that selfing is higher in the former than the latter. As expected, estimates of correlated mating substantially varied between the two seed orchard designs with 35 and 8% for the clonal-row and random seed orchards, respectively. Results from the mating pattern have demonstrated the presence of genetic quality differences of seed produced from the two seed orchard designs.

The present study demonstrated that both orchard designs produced similar seed yield, however, the genetic quality of the produced seed differed. If clonal-row seed orchard design will be considered for second- and/or advanced generation seed orchard, then effective pollen management such as SMP is required to reduce the selfing rate as well as correlated matings.

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ELEMENTS REQUIRED FOR XYLEM-SPECIFIC EXPRESSION ARE LOCATED DOWNSTREAM OF A LOBLOLLY PINE GENE

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Genetic engineering is likely to be a component of tree improvement activities in the next century. In order for genetic engineering of forest trees to be successful, the isolation of regulatory elements controlling when and where the transgenes are expressed will be needed. Although many promoters isolated from various crop or model plant species or from plant viruses are available, they may not always suit the needs of the forest biotechnologist. For various reasons, it may be important for an introduced gene or sequence to be expressed only in specific tissues or cell types or only in response to specific environmental cues. Occasionally, expression in other locations may be detrimental or even deadly to the plant. At other times, expression of a transgene in tissues where it is not needed may not be harmful, but it may not be useful either, and expression is therefore, a waste of the plant's resources. In some cases, regulatory elements that are satisfactory have been isolated from another species. However, elements regulating gene expression in one species are not always correctly recognized in another, especially when isolated from distantly related species such as the use of a monocot promoter in a dicot or an angiosperm promoter in a gymnosperm. Certain "constitutive" promoters have frequently been used in our early attempts at transformation of tree species. Although these promoters have sometimes been found to be valuable, some are protected by patents and cannot be used for commercial operations. Several tissue-specific promoters isolated from crop species are also protected by patents. For the reasons discussed above and others not mentioned, it is sometimes necessary to isolate regulatory elements from forest trees. Here, we will present the results of our attempts to isolate elements capable of conferring xylem-specific expression in loblolly pine for use in genetic manipulation of wood properties.

We initiated our search for xylem-specific regulatory elements by isolating clones of two genes preferentially expressed in differentiating xylem of loblolly pine. The cloning and characterization of PtX3H6 (Pinus taeda xylem) and PtX14A9 have been previously described (Loopstra and Sederoff, 1995). Transcripts of both are extremely abundant in differentiating xylem, much less abundant in needles and embryos, and non-detectable in megagametophytes. Transcript levels are similar in xylem isolated from the stems of 1, 2, and 10 year old trees and in earlywood and latewood. Expression is slightly lower in stems of very small seedlings (less than 10cM in height). Expression of neither gene is induced within two hours of wounding. We believe these two genes are the most abundant xylem-specific genes found in loblolly pine. PtX3H6 transcripts are more abundant than those of PtX14A9 and both are much more abundant than those of cinnamyl alcohol dehydrogenase and phenylalanine ammonia lyase.

Genomic clones were isolated for both PtX3H6 and PtX14A9 in order to obtain xylem-specific promoters. Sequence analyses of the PtX3H6 and PtX14A9 promoters have revealed some regions of interest. There are several short sequences (7 to 10 bp) found in both promoters. It is not known if this is due to chance or if they are conserved functional elements. Several 7 to 8 bp sequences are shared by the PtX3H6 or PtX14A9 promoter and that of GRP 1.8, a glycine-rich cell wall protein associated with vascular tissue in bean (Keller and Baumgartner, 1991). One 7 bp sequence is found in all three promoters as well as the promoter of GRP 1.0, another glycine-rich protein found in bean. In the GRP 1.8 promoter, the sequence is found at the 5' end of a negative regulatory element involved in vascular-specific expression. It is possible this sequence is involved in the xylem-specific

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expression of PtX3H6 and PtX14A9 but it is also likely that other elements are involved. Other sequences identified as regulatory elements in angiosperms are also present in the pine promoters. Primer extension analyses have been used to identify both transcription start sites. Both genes contain putative TATA boxes the appropriate distance upstream.

Fusions containing the PtX3H6 and PtX14A9 promoters and *uid*A, encoding GUS, have been tested in transient assays using microprojectile bombardments (Loopstra et al., 1995). A 3.6 kb PtX3H6 promoter and both 750 bp and 1250 bp PtX14A9 promoters were tested by bombardment of differentiating loblolly pine xylem, embryos, and megagametophytes. Promoters of both genes were found to be active in differentiating xylem. However, both the PtX3H6 and PtX14A9 promoter fusions resulted in stained foci on bombarded megagametophytes and embryos. The lack of xylem-specificity was originally attributed to the putative negative regulatory element. Fusions with both promoters were introduced into tobacco and hybrid poplar using *Agrobacterium tumefaciens*. The promoters were active in both systems although the PtX14A9 promoter was poor in tobacco. Again, we did not observe xylem-specific GUS expression. We attributed this to the fact that we were testing conifer promoters in angiosperms. A construct containing a 3.2kb PtX3H6 promoter was introduced into transgenic white spruce (*Picea glauca*) by Dr. David Ellis (U. Wisc. and BC Research) using microprojectile bombardment of somatic embryos and plantlets were produced. We believe this was the first time a pine sequence had ever been introduced into a conifer. Since spruce is much more closely related to pine than tobacco or poplar, we expected to see xylem-specific GUS expression but did not. All three lines produced had very different patterns of GUS expression. The combined results of the transient assays and the transgenic tobacco, poplar, and spruce led us to hypothesize that we were missing elements required for xylem-specificity and that they might be located in a position downstream of the transcription start site rather than in the promoter.

In many cases, a plant promoter and 1kb of 5' flanking sequence is sufficient to confer correct expression of a reporter gene in transgenic plants. There are however, exceptions to this. Leader introns, 5' flanking sequences further upstream than 1kb, internal elements, and 3' sequences have been shown to play important roles in controlling quantitative levels of expression, tissue-specificity, and inducibility. Sequences downstream of the translation stop codon have been shown to have regulatory properties in a few angiosperms. We now have evidence that sequences required for xylem-specific expression are found within the 3' untranslated or 3' flanking sequences of PtX3H6. Two different PtX3H6 promoter - *uid*A fusions were tested in transgenic tobacco. Both contained approximately 3.7kb of 5' flanking sequence. One construct contained the NOS (nopaline synthetase) terminator (pBI3H6GUS-5/N) and the other contained the pine PtX3H6 3' untranslated region including the terminator and approximately 1kb of 3' flanking sequence (pBI3H6GUS-5/3). GUS expression in transgenic tobacco was compared. In stems of plants transformed with the pBI3H6GUS-5/N construct (NOS terminator), GUS expression was high in all tissues. In stems of plants transformed with the pBI3H6GUS-5/3 construct (pine terminator and 3' flanking sequences), total GUS expression was greatly reduced and primarily restricted to the vascular tissues. Similar expression patterns were seen in petioles.

Further experiments are needed to determine if the 3' elements required for xylem-specific expression are located in the 3' untranslated or 3' flanking region. There are 7 sequences from 8 to 10 base pairs in length that are found within the 3' untranslated portions of both PtX3H6 and PtX14A9 that are candidates for a 3' silencer. The translated portions of the two genes are not particularly similar. We have not yet sequenced the PtX14A9 3' flanking region to determine if any conserved elements are found there also.

Based on the transgenic tobacco results and the conserved 5' and 3' sequences found, it is likely that sequences required for vascular-specific expression are probably located

upstream and downstream of the translated portion of these genes. In order to determine if 5' sequences are also required for xylem-specificity and to examine PtX14A9 expression, the following constructs are currently being tested in transgenic tobacco and poplar. Comparison of plants containing constructs with the same promoter but different terminators or the same terminator but different promoters will help determine the location of essential elements.

Construct	Promoter	Reporter gene	Terminator
pBI3H6GUS-5/N	PtX3H6	GUS	NOS
pBI3H6GUS-5/3	PtX3H6	GUS	PtX3H6
pBI3H6GUS-35S/3	CaMV35S	GUS	PtX3H6
pBI121	CaMV35S	GUS	NOS
pBI14A9GUS-5/N	PtX14A9	GUS	NOS
pBI14A9GUS-5/3	PtX14A9	GUS	PtX3H6
pBI14A9GUS-35S/3	CaMV35S	GUS	PtX14A9

It is possible that 3' elements controlling xylem-specificity do so not by controlling transcription but by affecting RNA stability. Run-on transcription assays are needed to determine the level of gene regulation. Within the 3' flanking region, there are two and one-half repeats of a 30 bp GT rich sequence and two repeats of a 28 bp sequence. Sequences within the repeats may be involved in RNA cleavage and processing.

Since the pBI3H6GUS-5/N construct resulted in higher levels of expression than the pBI3H6GUS-5/3 construct, it is expected that the sequences required for the high levels of expression observed are probably in the 5' flanking region. The PtX3H6 5' flanking region contains two pairs of repeats between approximately -830 and -570, one 63 bp in length and the other 36 bp. It will be interesting to determine if the 5' or 3' repeats contain regulatory elements. Once the required regions are identified, deletions will be used to more precisely locate elements required for xylem-specificity and high expression.

Certain patterns of GUS expression observed in transgenic tobacco containing either the pBI3H6GUS-5/N or the pBI3H6GUS-5/3 construct suggested that auxin may play a role in the regulation of PtX3H6. Plants containing both constructs had high levels of GUS expression in shoot apices and at nodes. Inhibitors of the plant growth regulators auxin, ethylene, and gibberellins were applied to loblolly pine seedlings. RNA was extracted from untreated seedlings and seedlings exposed to 1, 3 and 7 days of the inhibitors. Northern blot analyses were used to examine transcript levels. PtX3H6 transcripts decreased greatly following treatment with TIBA, an auxin transport inhibitor. Uniconazol, a gibberellin inhibitor, had no effect on transcript levels. Silver nitrate, an ethylene inhibitor, caused only a very slight decrease in PtX3H6 transcripts. PtX14A9 transcripts decreased moderately in response to the TIBA and greatly in response to the uniconazol and silver nitrate treatments. These experiments need to be replicated but it appears that the two genes are regulated by these growth regulators but to different extents.

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Keywords: Loblolly pine, xylem, regulatory elements

CHROMOSOME STRUCTURE AND MOLECULAR CYTOGENETICS OF SOUTHERN PINES

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Keywords: *Pinus elliottii*, *Pinus palustris*, *Pinus strobus*, molecular cytogenetics

Molecular cytogenetics is the application of the techniques of molecular biology to the study of chromosome structure and function. Molecular cytogenetics principally involves the application of fluorescent *in situ* hybridization or FISH, a technique that allows the localization of specific sequences on metaphase chromosomes and interphase nuclei. Other techniques, such as primed *in situ* amplification, *in situ* polymerase chain reaction, and total genomic *in situ* hybridization are also important. These techniques permit the correlation of genetic linkage groups and physical chromosomes. The techniques also allow the localization of repetitive sequences, and the localization of genes in gene families which often have greatly different copy numbers at each locus and intra locus polymorphism, making them difficult to map using other mapping techniques.

Physical mapping using fluorescent *in situ* hybridization (FISH) involves the preparation of spreads of intact chromosomes, pretreatment of these spreads to remove cytoplasmic materials, labeling and hybridization of probe DNA, and finally detection and visualization of sites of hybridization using fluorescent microscopy. Successful FISH experiments require preparation of slides with adequate numbers of metaphase chromosomes from diploid cells, well spread but with the complete complement, and without overlaying cytoplasm that blocks hybridizations. For preparation of pine chromosome spreads, radicles are collected from germinating seedlings and treated with colchicine to increase the number of metaphase cells. Root tips are then partially digested in a cell wall degrading enzyme mix (cellulase, hemicellulase, pectolyase, and pectinase) and the meristem dissected into a pool of acetic acid on a clean microscope slide. The dispersed meristem cells are then squashed onto the slide by standing on a glass coverslip placed over the slide. Before hybridization, chromosome spreads are treated with RNase and pepsin (a protease) to remove cytoplasmic material covering the chromosomes. Commercially available nucleotides labeled with fluorescent molecules (or molecules to which antibodies with fluorescent labels attached are available) are used to prepare DNA to be used as probe in FISH experiments. Labeled nucleotides can be incorporated into DNA using standard molecular biological techniques. This DNA and the chromosome spreads are heat denatured to separate the complementary strands of DNA. The probe solution is added to the slides so that during re-annealing the labeled DNA will base pair with the chromosomal DNA forming a hybrid strand. When viewed under a microscope with the appropriate filter sets, chromosomes, nuclei, and sites of hybridization can be clearly observed. Fluorescent dyes which stain nucleic acids, such as DAPI (4',6-diamidino-2-phenylindole, which stains A-T rich regions) and CMA

(chromomycin-A_3, which stains G-C rich regions)), are also used. These dyes provide information about overall base composition along the chromosomes and serve to counterstain the chromosomes, which is useful during analysis. Images of hybridization to chromosomes are captured on color print film. The negatives are then scanned and digitized, allowing computer analysis of pixel intensity along the chromosome median axis. Multiple chromosome spreads are analyzed and the data pooled to create ideograms, or diagrams of gene locations along chromosomes.

Applications of molecular cytogenetics techniques to pine is at an early stage of development. Patterns of fluorescent *in situ* hybridization to genes for the large and small rDNA subunits and fluorochrome banding patterns using CMA and DAPI have allowed all twelve pairs of chromosomes of slash pine to be identified and a standard karyotype proposed for pine (Doudrick et al. 1995). We are currently extending this karyotype to other pine species, including longleaf pine and white pine, allowing comparative analyses of patterns of hybridization. Preliminary results indicate that the 18-25S rDNA sites are highly conserved among the three species, with both intercalary and centromeric sites being observed in both longleaf and white pine, as in slash pine. Further analysis will be necessary to determine the conservation of exact distances along the chromosomes. The DAPI banding pattern of slash pine appears to be conserved in longleaf pine, but different in white pine as has been previously reported (MacPherson and Fillion 1981). DAPI negative bands have been observed in white pine but not DAPI positive bands. Both DAPI positive and negative bands are seen in longleaf pine, much as in slash pine. We are currently working to quantify these similarities and differences and to extend these results to other species.

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DEVELOPMENT OF TRANSGENIC YELLOW-POPLAR FOR REMEDIATION OF MERCURY POLLUTION

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Phytoremediation involves the use of plants to stabilize, reduce or detoxify pollutants, including heavy metals. A number of plants, known as hyperaccumulators, have displayed a tolerance for accumulation of high levels of some heavy metals and have generated considerable interest in their use for soil decontamination. However, hyperaccumulator plant species are typically slow-growing, possess low biomass and probably are of limited utility for phytoremediation. Engineering other plant species with the genes involved in hyperaccumulation may be one option for making use of this ability, but the genetic pathways for hyperaccumulation in plants are somewhat complex. By contrast, bacterial heavy metal resistance genes are assembled in discrete operons of small numbers of genes. The expression of bacterial heavy metal genes in transgenic plants could expedite the development of efficient phytoremediative species. Trees in particular are excellent candidates for engineering with heavy metal detoxification genes for use as phytoremediative crops, due to their large biomass, long lifetimes, and abundance of nonliving, woody tissues.

Aerobic bacteria may display broad-spectrum mercury resistance by virtue of a cluster of genes comprising the mercury resistance operon (Summers 1986). One gene of the bacterial mercury resistance operon, *merA*, codes for mercuric ion reductase, which converts Hg^{++} to elemental mercury, Hg^0. The *merA* gene provides a potential mechanism for mercury removal and detoxification using plant genetic engineering. Rugh et al. (1996) employed a directed sequence modification strategy to develop modified *merA* gene constructs for transformation and analysis in plant species. Overlap-extension PCR (OE-PCR) was used to create gene sequences having codon usage and nucleotide ratios more typical of highly expressed plant genes, though without altering the MerA enzyme sequence or structure. The bacterial gene was modified in a series of stepwise constructions to optimize the flanking regions and coding sequence of the gene, resulting in constructs with only the flanking region optimized (*merA0*), and with 9 percent or 19 percent of the coding region optimized in addition to the modified flanking regions (*merA9* and *merA19*, respectively). *Arabidopsis thaliana* plants transformed with *merA9* displayed high level Hg^{++} resistance (Rugh et al. 1996).

As discussed by Rugh et al. (1995), our long-term goal is to test the same modified *merA* gene in forest trees, which may someday be applied for actual remediation of mercury-contaminated sites. Using the technique developed by Wilde et al. (1992), *merA0, merA9* and *merA19* were each transformed into embryogenic yellow-poplar (*Liriodendron tulipifera*) cells as inserts in plasmid pVSTI. The same plasmid without inserts of the modified *merA* genes was used to generate transformed controls. Bombarded cells were selected on 100 μg/ml kanamycin and kan-resistant colonies of PEMs began to appear after about 2 months. Between 30 and 45 independent putatively transformed PEM colonies were generated for each *merA* construct. Small clumps of putatively transformed PEMs were tested for survival and growth on semisolid medium with 25 or 50 μM $HgCl_2$. Many mercuric ion-resistant lines were found, some able to withstand up to 100 μM Hg^{++}. After one year, however, many of the putative transformants were apparently no longer Hg^{++}-resistant. The loss was especially striking among *merA0*-transformed lines, compared to those transformed with *merA9* and *merA19*. Genomic DNA PCR was used to detect *merA* transgenes in putative transformant yellow-poplar PEMs. The percentage of lines confirmed by PCR to contain *merA* transgenes that were Hg^{++}-resistant was determined for each merA construct set. This percentage appeared to relate

well to the extent of plant-optimized gene modification for each transformed set. Methylation could have been responsible for this relationship, with the forms of the gene less optimal for plant expression tending to be methylated over time.

Suspension cultures were initiated from the nontransgenic source line, vector (pVSTI) transformed control, and lines transformed with *merAO, merA9* and *merA19*. PEMs grown in suspension were size fractionated as described in Merkle et al. (1990) and plated on basal medium with or without $HgCl_2$ to generate populations of somatic embryos. Only one line transformed with *merA19* produced mercuric ion-resistant embryos from fractionated PEMs following plating on medium with 25 or 50 μM Hg^{++}. Embryos from this line could also germinate on germination medium with up to 50 μM Hg^{++}.

We used a mercury vapor analyzer to measure activity of the MerA enzyme in regenerated *merA19* plantlets and untransformed control plantlets. Germinants were planted in 15 mls of half-strength germination medium gelled with 1.5% low melting point agarose, while the medium was still molten, allowing the medium to gel around the root system of each germinant. Tubes were attached via a sampling port to the mercury vapor analyzer and the headspace in each tube was sampled every 12 hours for 6 days. As the end of the mercury evolution assay, two plantlets of each genotype were removed from the tubes and analyzed for mercury content. Hg^0 release rates were dramatically different between the *merA19* plantlets and control plantlets, with *merA19* plantlets displaying up to ten times the Hg^0 evolution rates of control plantlets. Analysis of *MerA*-yellow-poplar plantlets confirmed that they also accumulated less mercury in their tissues relative to controls.

These preliminary results indicate that the modified merA genes can confer mercuric ion resistance and detoxification on yellow-poplar trees. We are currently preparing experiments to examine the ability of *merA*-yellow-poplar plants to extract and detoxify ionic mercury from prepared soil mixtures as a model for actual site remediation.

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EFFECTS OF REGENERATION METHODS ON GENETIC DIVERSITY IN SHORTLEAF PINE (*Pinus echinata* Mill.)

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Continuing demand for forest products, the increasing demand for use of forested areas for non-traditional purposes, and the general increase in awareness of the need for maintaining landscape diversity and biodiversity, wildlife conservation, protection of old growth forests, control of ecodegradation and global climate change has put a complex array of often conflicting demands, priorities and conditions on forest managers. The choice of suitable management strategies applicable to the climatic, political and public situation of the forests under their care has become exceedingly difficult. Therefore, an evaluation of within-species genetic diversity of natural stands compared to that of stands regenerated by various management schemes would help in understanding man's effect on these stands, and may suggest suitable management strategies. This study examined the effect of regeneration methods on genetic diversity in shortleaf pine (*Pinus echinata* Mill.) by quantifying the changes in genetic composition of shortleaf pine stands following harvest by monitoring changes in allele number and frequency at heterozygous loci over time. The study involved two steps. The first step examined isoenzyme variation and genetic structure in natural populations of shortleaf pine. The second step examined genetic variation in two stands and it's changes due to different regeneration management systems.

The first part of the study, reported in detail by Raja *et al.* (1997) examined seed from populations representing 15 geographic locations covering much of the natural range of shortleaf pine using 23 enzyme systems and 39 loci. Populations were polymorphic (p) at 87.2% of the loci, had 2.18 alleles (A) per locus and 2.35 alleles per polymorphic locus (A_p). Mean expected heterozygosity (H_e) was 0.194 and mean observed heterozygosity (H_o) was 0.174. Western populations had a higher p, higher A, similar A_p and a higher H_o and H_e than eastern populations. Interpopulation genetic variation was 9% percent, meaning 91% of the genetic variation in shortleaf pine resides within populations. Interpopulation gene flow was relatively high and explains the small interpopulation genetic variation in the species. Shortleaf pine populations exist in naturally outcrossing random-mating populations and have a relatively large amount of natural variability.

For the second part of the study, seed from 48 trees was collected from each of two shortleaf pine stands in the Ouachita Mountains near Mt. Ida, Arkansas. These 40-acre 60-80 year old stands contained shortleaf pine and a mixture of other deciduous species, predominantly *Quercus* and *Carya* species. Each stand was subdivided into quarters of approximately equal area arranged perpendicular to the elevation gradient and each quarter further subdivided into thirds along the elevation gradient, as part of a large ecosystem management research study on the Ouachita and Ozark National Forests in west-central Arkansas and eastern Oklahoma. A plot center was marked in each of the 12 plots and 4 healthy trees with abundant cones were selected from each plot for seed collection in this study. Seed-tree and single tree selection harvest / regeneration systems were applied to the two stands, respectively, following the first seed collection. The subsequent crop of seed representing genetic variation after management was then collected. The seed samples were assayed to detect changes in genetic variation due to management. Twenty-five seeds from each of the 48 trees from each stand for pre- and post-treatment were assayed for the 34 isoenzyme loci that were found polymorphic in stage one of this study (Raja et al. 1997). Fifty seeds each from the bulked seeds of Ouachita and Ozark seed orchards were also analyzed to represent artificial regeneration. Seed extraction and storage procedures, sample preparation, starch gel electrophoresis, enzymes staining and isoenzyme detection procedures followed protocols described by Raja et al. (1997).

Megagametophytes and embryos from each seed were scored for each locus. Identification of pollen genotype was accomplished by comparing megagametophyte and embryo data. Haploid pollen allele frequencies and diploid embryo genotypic frequencies were calculated. Allele frequencies for pre-treatment, post-treatment and artificial regeneration were compared with χ^2 tests (Snedecor and Cochran 1967, p. 250). When expected values were too small for χ^2 tests, Fisher's exact test was used (Sokal and Rohlf 1981, p. 740). Genetic diversity was estimated by percent polymorphic loci '*p*', mean number of alleles per locus '*A*' and mean number of alleles per polymorphic locus 'A_p'. Diploid embryo data from each stand were pooled for pre- and post-treatment to calculate the observed (H_o) and expected (H_e) heterozygosities, and the fixation index using the formula $F = 1 - H_o / H_e$. H_o and H_e were calculated using the BIOSYS-1 computer program (Swofford and Selander 1981).

Both natural regeneration treatments resulted in higher genetic variation post-treatment, indicating a richer pollen cloud after management. Artificial regeneration showed much lower variation compared to both natural regeneration treatments. Frequency of alternate alleles increased at several loci in the seed-tree stand after treatment, which is an indication of less inbreeding or consanguineous mating. Single tree selection resulted in an increase in alternate allele frequencies at a relatively fewer loci and at some loci alternate allele frequencies decreased,

indicating that the treatment may result in more inbreeding than seed tree. Artificial regeneration showed a considerable increase in alternate allele frequencies at several loci and hence can be considered outbred. The above mentioned observations were confirmed by comparing H_o, H_e and F values for the two stands before and after treatment. The seed tree method resulted in a decrease in inbreeding, whereas single tree selection did not alter it. Artificial regeneration showed a value indicative of high levels of heterozygosity and outbreeding. Our results generally agree with the results of Neale and Adams (1985).

Acknowledgments

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PERFORMANCE OF SELECT SLASH PINE FAMILIES IN ARGENTINA AND USA

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Slash pine (*Pinus elliottii* var. *elliottii* Engelm.) is an important commercial species in the southeastern United States, not only within its natural range (Figure 1), but also in central Louisiana and eastern Texas, where it does not occur naturally, but has been widely planted. Slash pine is also successfully grown as an exotic in many temperate, sub-tropical and tropical areas of the world. It grows very well in Argentina. As early as 1971, over 46,000 ha of slash and loblolly (*P. taeda* L.) pine had been planted in Argentina (Barrett 1972).

Slash pine has been included in all the major tree improvement programs in the southeastern US, including the US Forest Service's Southern Region (R-8) Tree Improvement Program. This study documents the growth of progeny from trees selected for the R-8 Tree Improvement Program in Argentina.

A progeny test of 92 open-pollinated slash pine families was established at three locations in the Mesopotamia of Argentina: Puerto Esperanza, Misiones (26.2 °S); Bella Vista, Corrientes (28.4 °S); and Concordia, Entre Rios (31.4 °S). These latitudes are approximately equivalent to Miami, FL, Orlando, FL, and Savannah, GA, respectively, in the southeastern USA. Controlled-cross progeny of the same families were planted in 49 plantings in north Florida, south Mississippi and central Louisiana between 30 and 32 °N latitude (Figure 1).

All the ortets were selected in Mississippi and Florida by personnel of the R-8 tree improvement program also between 30 and 32 °N latitude except for 4 sources from Marion County, FL from 29 °N. Of the 43 families from Mississippi and 49 families from Florida, 41 were probably from plantations established by the Civilian Conservation Corps in the 1930's. The check lot for the US plantings consisted of several woods-run bulk sources. The check lot for the Argentina plantings was collected from a local (land race) slash pine seed orchard.

After 5 years in the field, the plantations in the US averaged 3.5 m in height, those in Argentina averaged 6.9 m. Height in the northernmost planting in Argentina, Puerto Esperanza, the most tropical location, averaged 7.5 m, whereas the height in the southernmost planting, Concordia, the most temperate location, averaged 6.0 m. Height of the trees in the intermediate location averaged 7.0 m.

Since none of the 49 plantings in the USA contained a substantial proportion of the total number of families, the Best Linear Prediction (BLP) process (White and Hodge 1989) was used to estimate overall heights and compute genetic gains.

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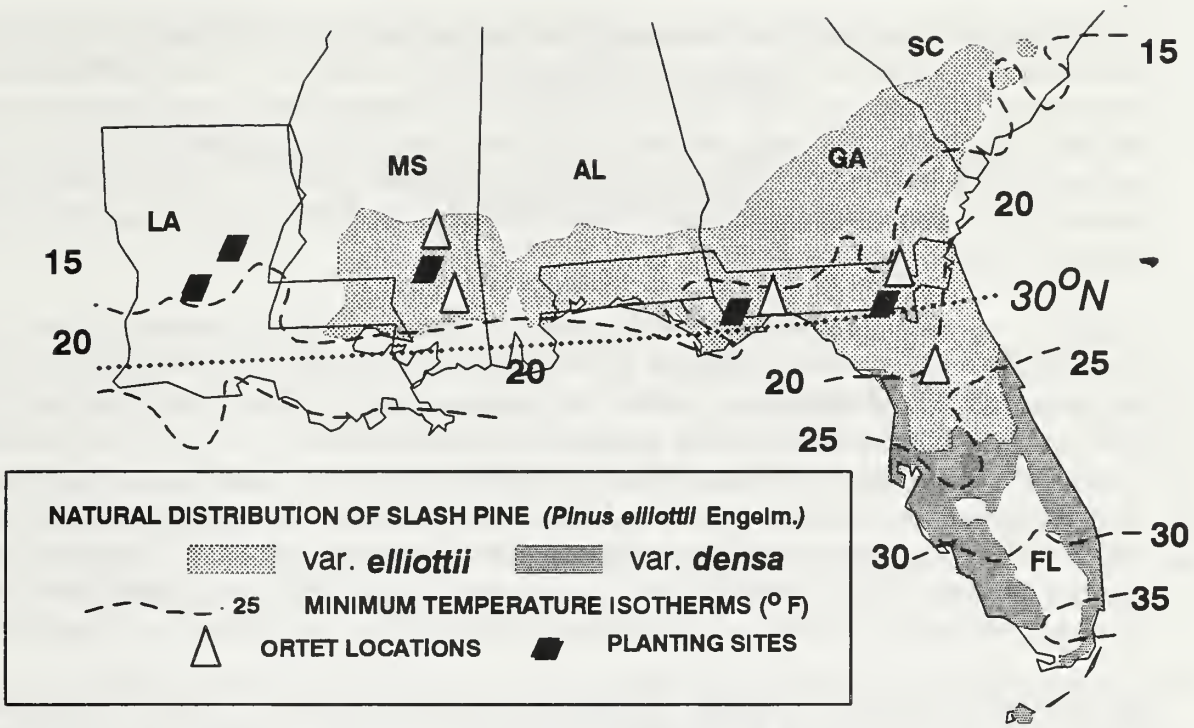

Figure 1. Map of the southeastern United States showing the natural distribution of slash pine and the location of seed sources and progeny tests.

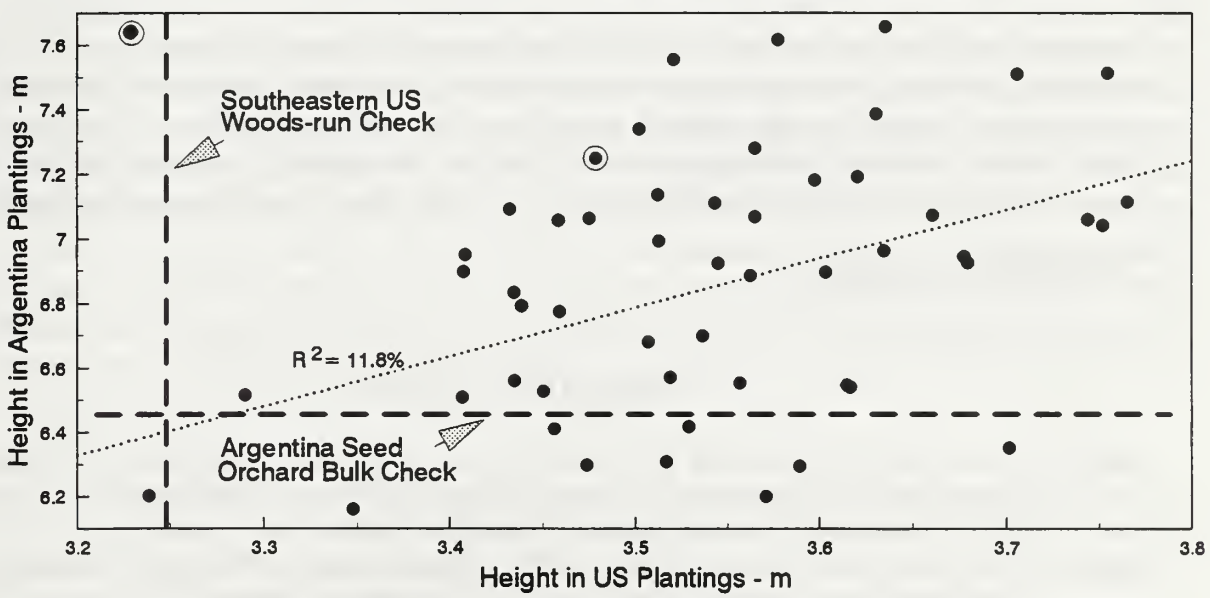

Figure 2. Five-year height growth of slash pine families grown in the Mesopatamia of Argentina compared to growth of the same families in the Southeastern United States. Families from Marion County, FL, are circled.

Height growth of the families in the USA plantings was only weakly correlated with growth of the same families in Argentina ($r=0.27$, $p<0.04$) (Figure 2). The correlation was strongest between height in the USA and height in the planting that is presumably the most similar climatically, the more temperate southern planting in Concordia ($r=0.42$), and weakest with the most tropical planting, in Puerto Esperanza ($r=0.17$). The correlation was intermediate between family means in height between the USA plantings and the central planting at Bella Vista ($r=0.26$).

In spite of the poor correlation of performance in Argentina with performance in the USA, substantial genetic gains were obtained in both locations. The average genetic gain in 5 year height in the USA plantings was 14%. The average genetic gain in Argentina averaged 25%, even though the check lot was a local seed orchard source. There was some evidence for provenance effects in the present study. Conventional wisdom states that geographic variation within typical (var *elliottii*) slash pine is negligible (Snyder *et al* 1967), but studies where sampling was intense show substantial variation (Squillace 1966). A series of slash pine provenance tests in Argentina have shown that growth varied with minimum temperature at the source (Data from Barrett (1974), re-analyzed by Schmidtling[4]).

The families in this study originate from about the same latitude just north of 30th parallel except for the four families from Marion County, FL (Figure 1) which are from further south. The USA plantings are all north of Marion County by at least 1° in latitude. The Marion County families average only 3.35 m in height in the USA plantings versus the overall mean of 3.52 m, indicating a possible mal-adaptation to the colder climate. In contrast, the Marion County selections average slightly taller in the Argentina plantings, 7.33 m versus the overall average of 6.86 m.

Using plus-tree selections from the USA for planting in Argentina appears to be a good strategy. Selections from the more southern range of var *elliottii*, but north of the transition to var *densa*, might have the greatest possibilities for use in Argentina. Considering the more tropical nature of the planting sites in Argentina, the *elliottii X caribaea* hybrids which are commonly used in subtropical regions of Australia, may have even greater potential.

[4] Manuscript in preparation.

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MAPPING QUANTITATIVE TRAITS FOR WOOD DENSITY IN LOBLOLLY PINE

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EXTENDED ABSTRACT

We have been focusing on identifying the quantitative trait loci (QTLs) which control wood property traits in loblolly pine (*Pinus taeda* L.). Previously GROOVER *et al.* (1994) mapped QTLs for wood specific gravity (WSG) using data from 177 progeny of a three-generation pedigree. This pedigree was constructed from two grandparental pairs which both displayed divergent values for WSG (scored as an average value of WSG for the first 8 years of growth). A conservative single-factor ANOVA approach (EDWARDS *et al.*, 1987) was used to detect five QTLs on five different linkage groups at $p \leq 0.05$ (GROOVER *et al.* 1994).

More recently KNOTT *et al.* (in press) extended the methods of HALEY *et al.* (1994) and VISSCHER and HALEY (1996) to fit an outbred model. These methods were used to re-analyze the WSG data by fully utilizing the three-generation structure of this outbred pedigree. These analyses combine information from a number of linked markers to provide the most powerful test for the presence of genetic variation. At regular genomic intervals the probability of an offspring being each of the four possible genotypes is calculated (HALEY *et al.* 1994). These genotypic probabilities were combined to consider the difference in effect of the alleles inherited from the maternal parent (ie, the difference in effect between the allele that originated in the high-WSG grandparent and that for the low-WSG grandparent), the paternal parent and an interaction term (which provides information about the deviation from additivity of the four alleles). The phenotypes are then regressed onto these probabilities using least squares analyses (HALEY and KNOTT 1992; HALEY *et al.* 1994). These regression methods are relatively simple and compare favorably to maximum likeliehood methods, thereby allowing the analysis of more complex (and potentially more realistic) genetic models (HALEY and KNOTT 1992; HALEY *et al.* 1994). Therefore, in addition to identifying regions that harbor a single QTL, we can now estimate the effects of whole linkage groups, test for the presence of polygenic variation and also test for the action of several linked QTLs (VISSCHER and HALEY 1996; KNOTT *et al.* in press).

In the re-analysis of WSG in KNOTT *et al.* (in press), a sex-average map consisting of 12 linkage groups was constructed from 171 progeny and a subset of 119 genetic markers (selected from 316 available makers based on informativeness and even spacing). Analyses

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were performed for one and two QTLs per linkage group. Two levels of significance were reported for each analysis (Lander and Kruglyak 1995). A "suggestive level" is where one significant result is expected by chance in a genome-wide analysis. A "significant level" is where a significant result is expected to occur 0.05 times in a genome-wide analysis. The genome-wide analysis includes 12 independent tests (one for each linkage group). Therefore, $p \leq 0.083$ and $p \leq 0.0043$ (using Bonferoni's correction) would be required for each linkage group to obtain a suggestive and significant genome-wide level, respectively.

Linkage groups 3, 13 and 14 contain a single QTL attaining the suggestive level of significance and linkage group 7 contains a single QTL at the significant level. The effect of the QTL on linkage group 7 accounts for 8% of the residual variance in the progeny. The estimates of the effect of this QTL suggest that both parents are heterozygous at this locus and that the alleles are not additive. Linkage groups 2 and 3 both showed evidence at the suggestive level for two QTLs. In each case, the allele with a relative positive effect for one QTL originated from the same parental gamete as the allele with the negative effect at the second QTL (thereby cancelling the effects of each other under a single QTL model). The conclusion of these analyses is that the current data does not support the presence of single QTLs with large effect for WSG, although there is evidence for QTLs with small effect (KNOTT *et al.* in press).

Recently, new phenotypic trait data has been collected for this pedigree to study components of wood specific gravity (eg, earlywood SG, latewood SG, a weighted average of early- and latewood SG, percent volume of latewood, and ring width). This data was collected ring by ring for years 3-11. The results from a preliminary single QTL analysis of these new traits were compared to those of KNOTT *et al.* (in press). For each of the following QTLs from this preliminary analysis, the genetic effects for the mother, father and interaction term were in the same direction as those found in KNOTT *et al.* (in press). On linkage group 3 for both latewood SG and weighted average SG at ring 11, a single QTL attaining the suggestive level of significance was found within 30 cM of that found in KNOTT *et al.* (in press). Both studies detected a single QTL (for earlywood SG at ring 4 which accounted for 10% of the residual variation in the preliminary study) at the significant level on linkage group 7, although they are at opposite ends of the linkage group. However, at ring 10, each of the five new traits showed evidence for a single QTL on linkage groups 13 and 14. On linkage group 14, four of the five new traits exceeded the significant threshold and each accounted for approximately 13% of the residual variance. For these two linkage groups, the new traits reside within 14 cM of those found in KNOTT *et al.* (in press). Although other single QTLs were detected at the suggestive level for this preliminary analysis (results not discussed), the results from this study and those of KNOTT *et al.* (in press) show that for the most part the findings are comparable among these different but related traits.

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CLONAL FORESTRY, HETEROSIS AND ADVANCED-GENERATION BREEDING

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Extended Abstract. Clonal forestry has been practiced in *Populus* for hundreds of years; more recently, techniques in vegetative propagation have made it possible to consider clonal forestry for many conifers. Clonal forestry offers several advantages over traditional seedling establishment practices, including planting stock and product uniformity, alternative disease management strategies, and the potential to capture greater amounts of the genetic variation. To date, our breeding strategies have not focused on clonal commercial plantations, even in *Populus*. Most current tree breeding programs rely on methods developed by corn breeders or livestock breeders, where breeding values and progeny tests are used to identify superior parents which are subsequently placed into open-pollinated seed orchards. Progeny are then collected as seeds and distributed to commercial plantations. Alternatively, with clonal planting stock, breeding programs need to develop superior individuals. Traditional advanced-generation breeding designs are based on the primus of population improvement, i.e., advancing the mean of the commercial population over that of the P1 generation. If clonally propagated genotypes are to be deployed operationally then the breeding design needs to be modified to maximize the probability of finding a superior individual. To maximize genetic gains per unit effort breeding strategies should be designed to increase recombination and within-family variance. Statistical theory indicates that means and variances are independent. Even the number of progeny included in present progeny tests are based on optimizing estimates of the mean, not estimates of the variance (30 individuals per family vs. 300-500, respectively). Thus, recurrent selection schemes utilizing factorial or diallel mating designs do not necessarily increase the probability of finding a superior individual.

A second property of clonal forestry that stands apart from seedling propagated forestry is the opportunity to capture heterosis. Heterosis, or hybrid vigor, is defined as the expression of a superior phenotype within the progeny of a single cross relative to either parental genotype (Shull 1911). Heterosis has been demonstrated in everything from chickpeas to chickens. It certainly occurs in inter- and intra-specific crosses and is the rule in most *Populus* breeding programs. In rice and corn, heterosis has been associated with gain yield, disease resistance, plant habit, ripening date, leaf orientation, and many other traits. In *Populus* heterosis has been documented in leaf size, leaf angle, growth rate, water-use efficiency, and branch habit (Bradshaw and Stettler 1995). It is reasonable to expect that heterosis occurs in conifers, particularly if it is the indirect result of mutational load (Franklin 1969, Tuskan and Wiselogel 1985, Strauss 1986). Heterosis occurs in two basic forms--dominance and overdominance. In rice the predominant form of heterosis is dominance; in corn it is overdominance (Stuber *et al.* 1992); in *Populus* both forms have been reported (Bradshaw and Stettler 1995, Li and Wu 1996). Heterosis attributed to dominance is caused by the sum of all dominant alleles at all loci affecting the desirable phenotype. The combination of favorable alleles from alternate parents conveys superiority to the progeny (i.e., AaBbCc . . .). It is this masking of mutational load that creates heterosis (Klekowski 1988). Under dominance heterosis it is possible to create superior, true-breeding genotypes (i.e., AABBCC . . .). Heterosis attributed to overdominance is caused by the

expression of both alleles in the heterozygote. Under overdominance heterosis all alleles manifest themselves and thus have an associated fitness. Classic examples of overdominance include circumstances where a fluctuating environment allows each alternate form of a gene to be favored, e.g., multimeric polypeptides formed from alleles adapted to high and low temperature. In the F1 generation, the heterozygote appears superior to either parent for either dominance or overdominance heterosis. Thus, the decision on which advanced-generation breeding strategy should be employed will depend upon the form of heterosis expected in the breeding population. If heterosis is attributed to dominance then a simple recurrent mating design will ultimately lead to superior genotypes (Simmonds 1981). Selfing and sib-mating in the breeding population could also be used to accelerate progress toward such genotypes. If heterosis is attributed to overdominance then a reciprocal recurrent mating design works best (Simmonds 1981). Here the clonal planting stock is derived from crosses between the reciprocal populations containing homozygous breeding populations that are used to create production clones with maximum heterozygosity.

Until the extent and form of heterosis is known, maximum genetic gains in a clonal breeding program may not be possible. For example, heterozygosity and thus overdominance heterosis will be lost during repeated simple recurrent selection and mating. Molecular markers can be used to discern the extent and form of heterosis in the breeding population. Genomic mapping and QTL analysis can provide indirect indications of the number of type of heterotic loci present in the mapped population. RAPD, STS, and SSR markers can be used to define the amount of genetic diversity in the breeding populations (Tsaftaris 1995). Experiments can then be designed to associate diversity with hybrid performance. If heterosis increases with increased genetic diversity among crossed parental lines then heterosis is attributed to overdominance. Alternatively, if heterosis increases with decreasing genetic diversity then heterosis is attributed to dominance. Numerous studies involving agricultural species have begun to assess heterosis using this approach (Maroof *et al.* 1997). Finally, molecular markers can be used to characterize gene function. Recent studies in corn indicate that overdominance is due to regulatory genes that convey superiority as a result of heteromeric proteins that interact with both the promoter and enhancer regions of structural genes (Jones 1990, Tsaftaris 1995). Outside of long-term breeding experiments, molecular markers provide the best opportunity to characterize heterosis in forest tree species.

In summary, clonal planting stock offers many advantages to the forest products industry. Advanced-generation breeding strategies should be designed to maximize within-family variance and at the same time allow the capture of heterosis. Certainly there may a conflict in the choice of breeding strategy based on the trait of interest. It may be that the majority of the traits express heterosis due to overdominance. Alternatively, disease resistance is expressed as the lack of a specific metabolite or infection court then the homozygous recessive genotype may be the most desirable. Nonetheless, as the forest products industry begins to utilize the economic advantages of clonal forestry, breeding strategies will have to be optimized for these commercial plant materials. Here, molecular markers can be used to characterize the nature of heterosis and therefore define the appropriate breeding strategy.

Keywords: Mating designs, molecular markers/diversity, vegetative propagation.

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TRANSFORMATION AND EXPRESSION OF AN AUXIN BIOSYNTHESIS GENE IN TOBACCO AND HYBRID POPLAR

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The objective of this research is to increase wood/fiber production by elevated endogenous auxin in hybrid poplar trees transformed with an auxin synthesis gene. Indoleacetic acid (IAA) is a plant hormone that stimulates cambium activities and influence wood/fiber development (Roberts *et al.*, 1988). Phenotypic modifications have been described in transgenic plants over-expressing the *Agrobacterium tumefaciens* T-DNA genes responsible for auxin biosynthesis (Klee and Estelle, 1991). Two of the *Agrobacterium tumefaciens* Ti-plasmid T-DNA genes have been shown to encode a biosynthetic pathway for IAA production that is different from plants. The *iaa*M gene, which encodes an enzyme converting tryptophan to indole-3-acetamide (IAM) (Thomashow *et al.* 1986) is subsequently hydrolyzed to IAA by the gene product of *iaa*H. Although IAM is not a standard intermediate in plant auxin biosynthesis, the expression of both *iaa*M and *iaa*H is not normally required for synthesis of IAA. Research in petunia suggests that overexpression of *iaa*M alone leads to high IAA production via hydrolysis (Klee *et al.*, 1987). We therefore constructed two gene cassettes designated as pZKY99 and pZKY100, which contain 19SCaMV-*iaa*M, nos-*npt*II and 35SCaMV-GUS within the T-DNA region. The only difference between the two plasmids was the reversed transcription orientation of the *iaa*M gene. The combination of GUS reporter gene and *npt*II selectable marker with *iaa*M gene in T-DNA region makes the constructs uniquely different from other available IAA-gene containing constructs (Klee *et al.*, 1987; Sitbon *et al.*, 1992). The gene constructs were successfully introduced into tobacco, the model transformation system, and into hybrid poplar (*Populus trichocarpa* x *P. deltoides*, clone `53-246') using an *Agrobacterium tumefaciens*-mediated transformation system.

Optimization of tissue culture and transformation conditions

To date our focus has been on development of a reliable method of micropropagation of *Populus trichocarpa X P. deltoides*, and on the methods to improve subsequent transformation. To optimize the transformation conditions, three disarmed *Agrobacterium tumefaciens* strains, LBA4404, EHA105 and C58, containing the binary vector p35SGUSINT, were tested for their efficiency to infect '53-246.' Tested vector p35SGUSINT contains selectable marker *npt*II gene for kanamycin (Kn) resistance and GUS gene for histochemical localization of β-glucuronidase. Three independent transformation experiments all indicated that EHA105, an agropine type of supervirulence strain (Jin *et al.*, 1987), gave the highest GUS transient expression in 30-d-old calli, where up to 67% poplar tissues were GUS positive. Therefore, pZKY99 and pZKY100 were all transformed into EHA105 for further use in the transformation of IAA gene in hybrid poplar. Transformation conditions, such as Agrobacterial concentration, inoculation and cocultivation, were also determined.

Agrobacterial concentration: To examine the effect of bacterial titer on gene transfer efficiency, explants were inoculated with concentrations ranging from 10^5 to 10^{10} bacteria/ml for 3 hr.

Lower concentration of bacteria up to the level of 10^8 gave higher transformation efficiency. Thus, we routinely used a fresh overnight culture at a concentration of 10^7-10^8 bacteria/mL.
Inoculation: To study the influence of exposure time to bacteria, stem internodes were cut longitudinally and inoculated by immersion in 15-25 mL of a culture at a concentration of 10^8 cells/mL for 1, 2, 4, 20, 24 and 28 hrs. There was little difference in transformation efficiency up to 4 hr. Overnight exposure (20 hr) significantly reduced transformation efficiency. A 28-hr inoculation totally inhibited callus formation and shoot regeneration, probably due to the extensive bacterial contamination that inhibited explant growth. Three to four hr inoculation in a liquid culture with a slow agitation (50 rpm) was routinely used.
Co-cultivation: Inoculated explants were blotted dry with a sterile filter paper to remove excess bacteria and then transferred to a callus induction medium (CIM, WNA-see below + 0.5 mg/L 2,4-D) without any antibiotics for a co-cultivation period of 1-8 d. Up to 3 d of cocultivation resulted in the highest percentage of shoot regeneration on Kn-containing medium.
Plant regeneration and selection: After co-cultivation, explants were washed with WNA medium (Cole and Ernest, 1991) containing 250 mg/L of cefotaxime for 2 hr and then transferred to a shoot-induction-medium (SIM; WNA + 0.5 mg/L zeatin) supplemented with cefotaxime to eliminate bacterial carry over. Ten d later, 50 ug/mL of Kn was introduced to the SIM plates to select for transformed tissues. The frequency of adventitious shoot formation and embryo-like structures was determined 30-35 days after infection. Elongated adventitious shoots were then excised and transferred to hormone free, root inducing medium (WNA with 1% sucrose, 250 ug/mL cefotaxime) with 25 ug/mL Kn. Regenerated plantlets were transplanted to a sterile Bacto soil mix in a Magenta GA7 vassel when roots were appeared, and transferred to the pots in the greenhouse.

Expression of *iaa*M gene in tobacco plants

To overcome the lack of morphogenesis in putatively transformed hybrid poplar, pZKY99 and pZKY100 were tested for their T-DNA intergration and gene expression in tobacco transformation system. Approximately 40 putative transformants were rooted from Kn-containing RIM with a uniform, and unique morphological aberrations. Several morphological changes attributed to overproduction of auxin were apparent in transgenic tobacco, such as apical dominance, epinastic leaf growth and a massive adventitious root formation. These morphological changes were expressed more dramatically in pZKY99 plants than in those transformed with pZKY100, with changes so abnormal that none of pZKY99 transformed plants completed their life cycle. Histochemical analysis of β-glucuronidase (GUS) in the tissues indicated its strong expression in roots, stems and leaves of pZKY99 plants, but not in pZKY100 transformants. The result may be attributed to the pararell transcriptional orientation of *iaa*M and GUS in pZKY100 construct. In spite of predominant morphological modifications, Southern and Northern hybridizations using non-radioactive digoxigenin-labeling system did not reveal the integration and expression of *iaa*M gene in both pZKY99 and pZKY100 plants.

Biochemical validation of plant transformation

Biochemical verification of plant transformation with genes that regulate auxin overproduction requires that the transformed plants have higher levels of auxin (IAA) or its

precursor or catabolites (breakdown products), providing evidence of increases in pool size or its turnover. The successful insertion of *iaa*M gene produces an unambiguous biochemical signal, IAM, a compound that typically does not accumulate in higher plants, but a compoundthat, if present, can be converted by plants to IAA (Klee et al., 1987). The typical precursor of auxin in higher plants is indole-3-acetaldehyde. Therefore, the presence of IAM validates that plant transformation has indeed occurred. Auxin and its metabolites occur in low concentrations in plant tissues. We have established protocols to analyze IAA by capillary gas chromatography-mass spectrometry (GC-MS) using selectable ion minitoring. It was demonstrated that tobacco plants transformed with the pZKY99 gene cassette produce high levels of IAM, up to 36 μmol g^{-1} dry weight of stem and leaf tissues, confirming that genetic transformation had occurred. IAA levels were also increased by transformation but levels were still very low. Therefore, the primary effect of transformation was on the production of IAM, whereas mechanisms, such as turnover, catabolism, and conjugation, may function to keep the pool size of IAA relatively stable.

Hybrid poplar was also transformed with the same constructs and 75% of the calli were GUS positive, but the expression declined over the time. About 500 plantlets from independent transgenic plants of hybrid poplar have been regenerated and planted in soil. Although none of these plants showed positive reactions on GUS histochemical and enzymatic assays, further DNA and biochemical analyses are being evaluated.

Keywords: IAA, hybrid poplar (*Populus trichocarpa* x *P. deltoides*), genetic transformation

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Genetic Load of *Pinus patula* in Zimbabwe

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Abstract: Temperate and boreal conifers have higher genetic loads than other plants and most animals. Genetic load has not been previously reported for species indigenous to the Mexican-Central American center of Pinus species diversity. We report embryonic genetic load for *Pinus patula*, a Mexican species used as an exotic in Zimbabwe. The study is based on a Zimbabwe Forestry Commission inbred mating design (Burrows and Askew 1982) with five founders and their progeny, 12 parents, which were intermated at four levels of inbreeding. All founders are two to three generations removed from the original Mexican collections. *Pinus patula* has 6 to 8 embryonic lethal equivalents, a genetic load comparable to most temperate and boreal pines. This estimate is adjusted for non-genetic sources of mortality and assumes 1 to 2 archegonia. There is a linear decline in sound seed set with increased inbreeding (F = 0 to 0.5).

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GROWTH THROUGH AGE FIVE OF LOBLOLLY PINE CUTTINGS AND SEEDLINGS ORIGINALLY MATCHED USING THREE CRITERIA GROUPS

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If clonal forestry is to be practiced in the southeastern U.S. with rooted stem cuttings of native pines, it will be important to know how their growth compares with growth of similar seedlings. Procedural shortcomings that may have skewed results in comparison tests with other species have been summarized by Frampton and Foster (1993). Prominent among these shortcomings are unequal genetic backgrounds of the two propagule types and different sizes of the two types at planting. For example, Norway spruce (*Picea abies* (L.) Karst) cuttings were larger than seedlings at age 13, but the propagule types were from different families and the ortets that originally furnished the cuttings had been selected for vigor while the seedlings had not (Rouland et al. 1985). Alternately, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings were larger than cuttings after five years, but the seedlings also had been about 50% taller at planting (Ritchie et al. 1994). In addition, some seedlings had been derived from families that were not represented by cuttings, and the cuttings were composed of one-fifth the number of genotypes, or fewer. Of the few tests comparing cuttings and seedlings of loblolly pine (*Pinus taeda* L.), Foster (1988) found them to posses many similar size characters at age three. Although sizes were similar at planting and both propagule types were generated from the same five families, there were only ten total seedlings in the final comparison versus 121 cuttings.

The objective of this test was to compare rooted stem cuttings and seedlings of equivalent genetic background and initial size. Since the appropriate criterion for matching the initial size of seedlings with that of cuttings was not known, the test also examined five different criteria. Beginning December 17, 1991, dormant cuttings were rooted from 33-month-old seedling hedges that had been generated from four control-pollinated families of loblolly pine. Seven sets of seedlings were germinated at two-week intervals using the same seed lots, and were grown in tandem with the rooting of cuttings. The first set was germinated four weeks before cuttings were collected. All sets were produced in Hillsons Rootrainers containing a medium of peat moss, perlite, and vermiculite that had been mixed and loaded at one time. Cuttings were rooted and seedlings were grown in the same greenhouse, although the cuttings were within a shaded polyethylene mist chamber above a root-zone heating system and the seedlings were not. Twelve weeks after rooting was initiated on cuttings, all plants were gradually acclimated to outdoor conditions. Beginning three days before the test was to be field-planted, twelve cuttings were sampled from each family of rooted cuttings, and were evaluated for five criteria: stem height, stem diameter at 1 cm above the soil, stem dry weight, root dry weight, and total dry weight. The seven seedling sets were sampled similarly. For each family of rooted cuttings, a seedling set from the same family was then identified that most closely matched it based on one measured criterion. The process was repeated for each of the remaining four criteria. Since the seedling sets identified by some

criteria overlapped completely for all families, measured criteria clustered into three groups: (1) stem height and root dry weight, (2) stem diameter, and (3) shoot and total dry weight. Seedlings from these three criteria groups and the rooted cuttings were field planted as single-tree plots on April 10 and 13, 1992, using a randomized complete block design with 20 blocks. To maintain equal representation of the two propagule types, only one cutting was planted from any ortet since a seedling can represent only one genotype.

Trees were assessed annually for total height, diameter at breast height (DBH), diameter 15 cm above the soil (D15), lower stem taper, average crown diameter, number of stem growth cycles, and number of primary lateral branches produced. Diameter of the largest branch 1.8 to 2.4 m above the soil was measured at age five only. Data within each year were analyzed as a series of three 4 X 2 factorials (4 families X 2 propagule types); one analysis of variance for each of the three criteria groups. Family was considered to be a random effect and propagule type was a fixed effect.

In general, the family-by-propagule-type interaction was statistically significant only infrequently. For the 99 total analyses of variance conducted for all measured characters over five years, this interaction was significant at the 5% or 1% level in only five instances. Three of these occurred at age one and none occurred at age five. Therefore propagule types generally reacted similarly across the four families tested, and only their main effects will be presented.

Cuttings generally grew similar to seedlings that had been matched by at least one criteria group. For seedlings matched to cuttings based on initial height or root dry weight, tree height was similar for cuttings and seedlings by age four. These seedlings had been, however, significantly taller than cuttings at ages two and three. Seedlings originally matched by other criteria were taller than cuttings at all ages. DBH was similar for cuttings and seedlings matched by initial height and root dry weight by age three. DBH of seedlings matched by other criteria--including initial stem diameters 1 cm above the container's soil--were larger than those of cuttings through age five. D15 was always larger for seedlings than cuttings regardless of initial matching criteria. For all three groups of initial matching criteria, rooted cuttings exhibited less taper, or more cylindrically shaped trunks, than seedlings by age four. This was mostly due to lower stem diameters (D15) remaining larger in seedlings while DBH became more similar, especially with seedlings originally matched by height and root dry weight. By age three, crown diameters for cuttings were similar to those for seedlings that had been matched initially by height and root dry weight. Seedlings matched by initial shoot and total dry weights had crown diameters equivalent to those of cuttings at age five only; seedling crowns had been broader in earlier years. Seedlings originally matched to cuttings based on stem diameter had broader crowns for all years through age five. The number of annual growth cycles and the number of lateral branches produced annually on the main stem were generally similar for cuttings and seedlings matched by all groups of size criteria after age one. Diameter of the largest lower branch at age five was also similar between cuttings and seedlings from all criteria groups.

In summary, height, DBH, and crown diameter were statistically similar after five years for cuttings and seedlings matched to them by initial height and root dry weight. For this same group of matching criteria, however, D15 was larger and stem taper was greater for seedlings. Seedlings originally matched by initial stem diameter, or by initial shoot and total dry weights, were taller and had larger stem and crown diameters than cuttings. Number of growth cycles, number of primary laterals, and diameter of the largest lower branch were generally equivalent between cuttings and seedlings at age five regardless of matching criteria.

The general absence of significant family-by-propagule-type interactions suggests that good families identified by seedling progeny tests should perform equally well as rooted cuttings. Therefore these results support the testing and selecting of clones from any superior family. The test further indicates that rooted loblolly pine cuttings can grow similar to seedlings from the same families when they are matched by stem height or root dry weight, although stem diameters near ground level may be narrower for cuttings. If this difference results in measurable reductions in wood volume at rotation age, then genetic gains from clonal selections may be not be realized fully. Seedlings matched by other criteria were larger by several measures throughout the test, so this test also confirms that small differences in initial age or size may persist for at least five years.

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Keywords: rooting, vegetative propagation

Poster Abstracts

GENETIC BASIS OF FUSIFORM RUST DISEASE RESISTANCE IN LOBLOLLY PINE

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Abstract. Research using RAPD marker/disease phenotype associations to marker tag, map and thereby identify rust resistance genes in loblolly pine is in progress. In a study involving progeny from seven pine mother trees (families) inoculated with basidiospores from six single aeciospore rust isolates (SAIs), four different heterozygous resistance genes have been identified (one each) in four different mother trees. Apart from a single dominant resistance allele at a given locus in each of these four mother trees, other loci (in these four trees) with resistance potential, detectable with our isolates, appear homozygous recessive (lacking resistance). Five to 15 polymorphic RAPD markers now exist for the various resistance genes. A mother tree with dual resistance, two of the previously noted heterozygous resistance genes, has also been recognized in the study. Pine-rust interactions among the isolates and the five studied families clearly fit a gene for gene (complementary genetic system) model with four corresponding gene pairs. This model is expected to increase with regards to gene pairs. Marker and mapping data show that at least three of the four recognized genes reside in the same homologous linkage group in their respective mother trees and are therefore clustered. The position of the fourth gene is not yet determined. In the above noted dual resistance mother tree, the resistance alleles of the two different genes clearly reside on separate homologs in a single linkage group, reflecting their inheritance from two different parents. The observed clustering of resistance genes will impact resistance breeding. The search for additional resistance genes in loblolly and slash pine is continuing.

Keywords: *Pinus taeda*, *Cronartium quercuum* f. sp. *fusiforme*, RAPD markers

WHITE PINE CONE BEETLE POPULATION TRENDS IN NORTH CAROLINA AND TENNESSEE SEED ORCHARDS 1986 - 1997

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Abstract. Overwintering white pine cone beetle populations in dead cones were sampled from six Eastern white pine seed orchards beginning in 1986 and continuing through 1997. Two sampling schemes were used on each orchard. To collect cones 1. Predetermine 50 sq. ft. plots under individual trees, and 2. Random collections throughout each seed orchard. Generally, beetle populations peaked on the Edwards Seed Orchard in 1992 and 1995 while populations in Tennessee orchards peaked in 1992, 1993, and 1995. In comparison, population peaks were noted on the Beech Creek Orchard in 1988, 1993, and 1997. During the intervening years, populations were moderate or crashed. Very few beetles were found by either sampling technique on the Beech Creek or Edwards Orchards in 1994 while higher populations were discovered in the Tennessee orchards. These data suggest that in Tennessee during 1994, beetle populations were increasing. In 1995, extremely high beetle populations were observed on the Pickett Orchard. There were 76.6 live beetles per 50 sq. ft. sample. This was the largest overwintering beetle population recorded during the survey. In 1996 populations crashed to 0.5 live beetles per plot.

The Picket Seed Orchard in Tennessee had higher sustained overwintering beetle populations than other surveyed orchards. The Edwards Seed Orchard generally had the fewest overwintering beetles. Comparisons between sampling techniques indicate the 50 sq. ft. method tends to yield trends with less sampling noise making it easier to visually spot population fluctuations. For example, the random sample on the Beech Creek-North Carolina Source in 1997 showed a slight reduction in overwintering populations from the 1996-1997 sample periods, while the 50 sq. ft. sampling method showed a dramatic increase in beetle populations. Cone crop damage by mid-April, 1997 on the Beech Creek-North Carolina source was nearly 100 percent. No cone crop damage data were collected during many of the sample years, thus, we were unable to determine a correlation between cone crop damage and overwintering beetle populations.

Keywords: White pine, white pine cone beetle, seed orchard, *Conophthorus coniperda* (Schwarz), *Pinus strobus* L.

USE OF RAPD MARKERS TO INVESTIGATE THE EFFECTS OF POLLEN PROCESSING AND POLLEN STORAGE ON POLLEN VIGOR

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Abstract: Supplemental mass pollination (SMP) is a management technique often used in seed orchards when clonal phenology inhibits uniform male gamete contribution to the overall seed crop. To help ensure that each male in the orchard contributes equally to the crop, equal amounts of pollen from each clone in the orchard is used in a polymix. One of the major assumptions of the polymix approach is equal male gamete success. It has also been suggested that stored pollen, although viable based on *in vitro* germination experiments, may fail to produce filled seeds due to reduced vigor. In order to better understand the effects of processing and storage on male gamete success we utilized RAPD markers in combinations of fresh (F), processed (P), and stored (S) pollen from two males applied in equal amounts in a factorial array to a single female. Parent-specific RAPD markers were identified by screening 10-mer primers against eight megagametophytes from each of the parents [male clones 81028 (referred to as parent A) and 81069 (referred to as parent B), and one female clone 81077]. Due to their dominance, RAPD markers had to uniquely distinguish a single pollen parent from each of the other clones. Thirty-two primers were screened to identify pollen parent-specific markers which were not present in female clone 81077. Marker 167_2000 was found to be specific to, and heterozygous in, pollen parent A, and two markers 111_1400 and 122_0875 were specific to, and heterozygous in, pollen parent B. Given equal treatment and assuming equal male gamete success, we expected to find the pollen parent-specific RAPD alleles in 25% of our controlled cross progenies. A total of 192 progenies from each treatment combination: FAFB, FAPB, FASB, PAFB, SAFP, PAPB, PASB, SAPB, SASB were assayed for the presence of our RAPD alleles. Treatment FAFB suggested that fresh pollen from parent B was more successful (excess of 10%) in producing viable offspring than was fresh pollen from parent A. Using stored or processed pollen from parent B gave similar results (excess of 9% in FAPB, and 9% in FASB), indicating that processing and storage did not effect viability of pollen from parent B. There did, however, appear to be a positive processing and storage effect on pollen from parent A, resulting in an excess of viable offspring from parent A (excess of 10% in PAFB, and 3% in SAFB). Pollen from parent A was found to be more successful in producing viable offspring in all other treatment combinations, again suggesting some effect of processing or storage on pollen from parent A. Results of this experiment suggest that handling of pollen may influence its viability and that such effects may be different for different males in a pollen mix. Effects on pollen germination, pollen tube growth, or gametophytic selection might possibly explain these results. Further research is necessary to examine which if any of these factors are at work.

Keywords: RAPD, pollen processing, pollen storage, SMP, polymix

MOLECULAR MANIPULATION OF REPRODUCTION IN YELLOW-POPLAR

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Abstract. This project seeks to develop techniques for controlling the development of reproductive tissues in southeastern forest tree species, both for the production of trees that flower precociously, as well as for the production of sterile tree lines. A pair of homeotic genes that control floral development in *Arabidopsis*, *LEAFY* (*LFY*) and *APETALA1* (*AP1*), were placed under the control of a constitutive promoter and used to transform embryogenic yellow-poplar (*Liriodendron tulipifera*) cells. Transgenic yellow-poplar trees expressing the *LFY* gene are currently in the greenhouse; however, transgenics expressing *AP1* were not recovered. Work is also proceeding to transform yellow-poplar with the same genes under the control of an inducible promoter. Gene fusions of the cytotoxic *DTA* gene with the *LFY* and *AP1* promoters are under construction, and will be tested for their ability to eliminate flowering in yellow-poplar lines that flower precociously.

Keywords: *Liriodendron tulipifera*, *Arabidopsis,* precocious flowering, sterility, somatic embryogenesis

PROTECTION OF INDIVIDUAL TREES IN PINE SEED ORCHARDS FROM ATTACKS BY CONE AND SEED INSECTS

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Abstract: Two approaches for protecting individual trees are presented. A single tree spray system consisting of irrigation spray nozzles permanently mounted on PVC pipes was used to control the white pine cone beetle and reduced seed damage caused by the leaffooted and the shieldbacked pine seedbugs in an eastern white pine seed orchard in western North Carolina. A second installation of this single tree spray system also reduced cone attacks by the webbing coneworm on loblolly pines in a seed orchard in eastern North Carolina. Trunk implants of the systemic insecticide, acephate, protected individual loblolly pines from attacks by coneworms and seedbugs in a loblolly pine seed orchard in central Georgia. Criteria such as controlled breeding operations, genetic value, cone crop size, and inherent susceptibility to attacks can affect the need for protection and the allocation of control efforts for cone and seed insect pests on individual orchard trees.

Keywords: *Conophthorus coniperda*, *Dioryctria* spp., *Leptoglossus corculus*, Orthene, *Pinus strobus* L., *Pinus taeda* L., *Tetyra bipunctata.*

MICROPROJECTILE-MEDIATED GENETIC TRANSFORMATION OF LONGLEAF, LOBLOLLY AND EASTERN WHITE PINE

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Abstract. Embryogenic tissue cultures derived from immature zygotic embryos of longleaf, loblolly and eastern white pine were maintained in culture for up to two years, then bombarded with gold particles coated with a gene construct containing the GUS Reporter gene fused to an algal virus adenine methyltransferase promoter gene. Physiological expression of genetic transformation was identified in cultures of all three pine species within 48 hours, but not at 7 days. Expression of GUS activity was recorded in somatic embryonal heads of varied stages of development, suspensor cells and others of unidentified ontogeny. Collective expression of GUS in small clusters of cells suggested inheritance of the reporter gene through mitosis of the transformed progenitor. Multiple discrete sites of GUS expression were common in individual somatic embryos. This indicated densely associated multiple transformation events, which was enhanced by reducing the sample distance.

Keywords: Pinus *palustris* Mill., P. *strobus* L., P. *taeda* L., GUS, biolistics, gene promoter

SCREENING CDNA CLONES FOR USE AS PROBES IN FLUORESCENT *IN SITU* HYBRIDIZATION

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Abstract. Fluorescent *in situ* hybridization (FISH) is a promising technique for physical mapping in southern pines. The technique will allow mapping of cDNAs to known linkage groups even if the cDNAs are not polymorphic or are not segregating in the mapping population. Physical mapping will also allow determination and confirmation of synteny between maps in different pedigrees, and produced by different research laboratories.
Physical mapping is at an early stage of development in pines and is limited by a lack of good physical markers. For example, sequences of single to low copy number are needed to use as chromosome and chromosome arm markers. We are currently developing a methodology to screen candidate sequences for their utility as molecular cytogenetic markers. Known and anonymous cDNAs are being analyzed for sequence complexity and copy number using southern and dot blot analysis, with particular emphasis on identification of single and low copy number sequences. cDNAs, or complementary genomic clones, will then be used as probes for FISH in an attempt to correlate southern and dot blot data to FISH probe utility. cDNAs are also being sequenced to determine if they have homology to known genes. These cDNAs will also be used as probes in FISH to determine the amount of inference about sequence complexity, copy number, and distribution in pine, that may be drawn from knowledge of these characters in other angiosperm and gymnosperm species.

Keywords: *Pinus elliottii*, molecular cytogenetics, molecular genetics

GENETIC DIVERSITY OF LOBLOLLY PINE GROWN IN MANAGED PLANTATIONS: EVIDENCE OF DIFFERENTIAL RESPONSE TO CLIMATIC EVENTS

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Keywords: Pinus taeda, genetic diversity, adaptability, drought.

Adaptability of a forest tree species to a wide range of environmental conditions is partially dependent on the levels of genetic diversity found within the species. One approach to assess genetic diversity of a plant species is to examine long-term performance of provenances grown in common-garden experiments. This method allows direct comparison of performance of different families under actual field conditions. When conducted over a long period of time, the trees will likely be exposed to a variety of climatic conditions, both in terms of soil moisture levels and ambient temperature levels.

In this investigation, climatic conditions were found to be associated with differential growth patterns in provenances of loblolly pine (Pinus taeda L.) grown in common-garden trials, as measured by annual radial growth. Radial growth was assessed from annual rings extracted from forty-year-old trees growing in the Southwide Pine Seed Source Study. A sensitive analysis of variance revealed very small, but statistically significant, provenance interactions with climate over a thirty-year period.

Furthermore, differentiation between provenances appeared to be largest during periods of extreme climatic conditions. For instance, provenances originating from regions of relatively frequent drought events during the growing season were found to be more sensitive to drought, exhibiting reduced radial growth during a droughty period, compared to provenances from mesic regions, which largely maintained radial growth during a period of prolonged moisture deficit.

ADDITIVE GENETIC COVARIANCE FUNCTION FOR HEIGHT IN *PINUS TAEDA*

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Abstract:--Prediction of time trends in additive genetic (co)variances are valuable for estimation of gain over time and for choosing the optimum age of selection. A covariance function, which gives covariance of a trait at any two ages as a function of the ages, is predicted for height in Pinus taeda using data from six progeny tests of P. taeda planted in Eastern Highlands of Zimbabwe assessed at 1.5, 7.5, 9.5, 12.5, 13.5 and 22.5 years. The progeny tests involved factorial matings among 21 female x 7 male phenotypically superior parents selected from plantations in Zimbabwe and South Africa.

The additive genetic covariance matrix of tree heights at the six ages was estimated using individual tree model DFREML. Covariance functions were directly fitted to the covariance matrix using orthogonal polynomials with symmetric coefficients (Kirkpatrick et al. 1990). Full and reduced fits were used to estimate the most appropriate covariance function. A full fit, in which the number of orthogonal polynomials equal the number of ages estimates the coefficient matrix in such a way that the corresponding covariance function exactly reproduces the estimated (co)variances at the ages that were measured. These estimates, however, include the sampling errors. A reduced fit, in which the number of polynomials are less than the number of ages produces a smoothed estimate consistent with the sampling errors. When a reduced fit is used, the program requires the error covariance matrix. The error covariance matrix was estimated by the program after providing it with the phenotypic covariance matrix and assuming that a standard balanced half-sib breeding design with 7 male and 21 female parents, and 20053 residual degrees of freedom. The Lambeth model (Lambeth 1980) was used to estimate the phenotypic covariance matrix because phenotypic covariances between many of the ages at which the assessments were made were not possible to calculate. The justification of using the Lambeth model is that our data was consistent with the model and the model was derived from a larger sample size.

The best fit was a reduced fit (cubic polynomial). Although discrepancies between the observed values and those predicted by the covariance function were significant, the discrepancies were small. The first eigenvalue showed that the first eigenfunction accounted for 99% of the additive genetic variation indicating that there was substantial genetic variation for improvement of height growth at all ages. This also indicates that improvement at one age would result in improvement at all ages. In contrast, the small amount of genetic variation associated with all the other eigenvalues implies that selection to alter the shape of the growth curve will make very little or no progress.

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Keywords: Covariance function, eigenfunction, eigenvalue, genetic variation, Pinus taeda

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CHARACTERIZATION OF LACCASE GENES FROM SWEETGUM (*LIQUIDAMBAR STYRACIFLUA* L.)

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Abstract. A unique feature distinguishing vascular plants from lower plants is the lignification of their xylem cell walls. Laccase appears to be one of the enzymes involved in the final steps of lignin biosynthesis. In order to more fully elucidate the role of laccase in wood formation, efforts were made to clone the laccase genes from sweetgum, a southern hardwood species which is gaining in commercial importance. RNA from sweetgum cambium was used as template for the cloning of laccase genes via reverse transcription-polymerase chain reaction (RT - PCR). A 360 bp fragment was amplified using primers to a ligated anchor and a conserved copper-binding domain. The translated sequence was 42 % identical to the N-terminal 84 amino acids of an *Acer pseudoplatanus* laccase. Laccase mRNA was detected in xylem of sweetgum via northern blot analysis. Southern blot analysis of genomic DNA with 300 bp laccase cDNA fragment showed that sweetgum laccase was derived from a single gene. Using sequence from this cDNA fragment, a 457 bp genomic DNA fragment was cloned. This 457 bp fragment comprised 28 bp of downstream sequence and 429 bp of upstream sequence relative to a putative translational start site, and included proposed TATA and CAAT boxes. The prospective sweetgum laccase promoter region revealed homologies with several regulatory elements involved in the phenylpropanoid pathways of other species. Among these were two elements containing the consensus sequence ACCTA (Box L) and one element containing the sequence CCGT (Box A), both of which are putative *cis*-acting elements in the promoters of the cinnamate 4-hydroxylase (C4H) and phenylalanine ammonia-lyase (PAL) genes from *Arabidopsis*.

Key words: Laccase, promoter, RT-PCR, regulatory element, sweetgum

MICROSATELLITE VARIATION IN LOBLOLLY PINE CHLOROPLASTS

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Abstract. Organelle genomes can provide a different and useful perspective on genetic variation in organisms. A special feature of organelle genomes is uniparental haploid inheritance without recombination. In animals, high levels of genetic variation in mitochondrial genomes has made it possible to describe within species phylogenetic trees that could reflect population history and evolutionary divergence. However, plant organelle genomes have been less useful because plant mitochondria are more complex and chloroplasts have low levels of DNA sequence variation. PCR-based "microsatellite" markers from genomic DNA are based on simple sequence repeats (i.e., repeated sequences of single nucleotides or dinucleotides or trinucleotides) that often show high levels of variation in repeat number. In pine, the chloroplast genome (approximately 120 kb) is inherited through the pollen parent and contains 20 microsatellite regions. We found that the gene diversity of loblolly pine cpDNA microsatellite markers is relatively high, suggesting that cpDNA microsatellite markers could distinguish individuals and be useful for assessing paternity in seed orchards and natural populations.

Keywords: *Pinus taeda*, microsatellites, paternity, chloroplast

ULTRASTRUCTURAL CHANGES DURING EARLY DEVELOPMENT OF SOMATIC EMBRYOS IN LOBLOLLY PINE (*PINUS TAEDA* L.)

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Abstract. Ultrastructural changes of somatic embryos at early stages were studied in loblolly pine (*Pinus taeda* L.). Proliferation and maturation media of Gupta and Pullman (1991) have been used to obtain embryogenic tissue and somatic embryos, respectively. Some degenerating and declined suspensor cells were observed in well-maintained embryogenic tissues. This may explain why the embryogenic cultures have to be subcultured frequently and sometimes loses embryogenic ability. In stage 1 embryos, starch granules started to accumulate in plastids, but lipid bodies showed no significant increase. In comparison to the stage 1 embryos, embryos at stage 2 contained increased number of lipid bodies. Cytoplasm of embryonic cells was also richer in free ribosomes, which may be an early sign of storage protein synthesis. Vacuoles in stage 1 and 2 embryos were larger and/or more numerous than that of embryonic cells in maintained embryogenic tissue. Lack of osmoticum in maturation medium was suspected as one of the potential causes of vacuolated embryonic cells with no protein accumulation, which has a very important role in somatic embryos.

Keywords:

SURVIVAL AND GROWTH OF SELECT LONGLEAF PINE FAMILIES INOCULATED WITH PISOLITHUS TINCTORIUS AND TREATED WITH BENOMYL

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Abstract: The objective of this study was to determine the effects of *Pisolithus tinctorius* (Pt) ectomycorrhizae and benomyl on the survival and growth of select longleaf pine families. A total of four families varying in brown-spot resistance and growth potential were compared in this experiment: (1) Abe x wind; (2) 27-168 x wind; (3) 30-142 x wind; and (4) 5-77 x wind. Two of the families (Abe x wind and 27-168 x wind) have shown good brown-spot resistance and rapid early height growth, and the other two families (30-142 x wind and 5-77 x wind) are more susceptible to brown-spot and are slow growers. Nursery beds were prepared by treating in the spring with methyl bromide two weeks prior to inoculation and sowing of seeds, which was done on May 15. The Pt treatments were: (1) mycelium-vermiculite inoculum or Pt spore pellets applied 1-2 in. deep in a 2 in. wide band, or (2) no inoculum. The four families were completely randomized into five blocks for each treatment at sowing time. Seedlings were all lifted just prior to planting in January of the following year. At that time, a randomized sample of five seedlings from each family/treatment/block combination were evaluated for percent Pt. Only inoculated seedlings with >10% Pt infection were used. Seedlings were divided into pairs at the time of lifting. At planting, one seedling of the pair was root-treated with a 5% a.i. benomyl-clay mixture, while the other received plain clay. The planting consisted of 5 blocks x 2 Pt treatments x 4 families x 2 root treatments x 5 seedlings for a total of 400 seedlings. Survival, total height, and percent brown-spot infection data were collected five years after outplanting. Analysis of the survival data did not suggest any significant interactions among the main effect variables. The benomyl treatment was found to significantly increase survival (Prob.>F=0.0231), 78% survival with benomyl versus 68% without benomyl. A significant family effect was also detected (Prob.>F=0.0474), with 74%, 80%, 75% and 63% survival for the four families, respectively. Analysis of the height growth data did not suggest any significant interactions, however, three of the main effect variables were significant. Family effects were highly significant (Prob.>F=0.0001), with mean heights for the four families of 127 cm, 183 cm, 109 cm,and 169 cm, respectively. The effect of the Pt treatment was significant (Prob.>F=0.0105), seedlings treated with Pt had an average height of 131 cm whereas seedlings not treated with Pt averaged 162 cm. The effect of the benomyl treatment was also highly significant (Prob.>F=0.0001), seedlings treated with benomyl had an average height of 198 cm whereas seedlings not treated with benomyl averaged 89 cm. Analysis of the brown-spot severity data identified a significant three-way interaction among the three main effect variables family*Pt*benomyl. In our poster we graphically present this data, point out some of the underlying interactions of the Pt and benomyl treatments on the various longleaf pine families, and discuss this data in terms of previous rankings of these families for brown-spot resistance and rapid early height growth.

Keywords: *Pinus palustris* Mill., *Pisolithus tinctorius*, benomyl, brown-spot

MONOTERPENE COMPOSITION IN LOBLOLLY PINE ATTACKED BY SOUTHERN PINE BEETLE

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Abstract. Exudation of oleoresin is one form of defense that pines have against attack by the southern pine beetle (SPB; *Dendroctonus frontalis* Zimm.; Coleoptera:Scolytidae). This resin flow functions primarily to expel the beetles and associated fungi vectored by the beetle from inside the tree. In addition, monoterpene fractions of the resin can be toxic to both the beetle and fungi, and therefore, the resin flow also acts as a chemical barrier. In an SPB attack, there are individual trees that are missed or for some reason not attacked by the beetles. These trees may possess some resistance or repellant that protects these trees from the beetles. In this study, we compared the monoterpene composition in trees attacked and missed during an SPB outbreak in Gainesville, Florida during 1995 and 1996.

Cortical samples were collected from 117 loblolly pine trees (*Pinus taeda* L.) growing in three separate stands that were being actively attacked by SPB. Within a stand, trees that were attacked (as evidenced by numerous pitch tubes) were sampled as well as those adjacent trees that were missed (lacking any pitch tubes). In total, 59 attacked trees and 58 missed trees were sampled and headspace analyzed with gas chromatography. The chemical composition of oleoresin differed significantly between attacked and missed trees. Missed trees showed generally higher percentage of all monoterpenes including α– and β–pinene, myrcene, limonene, β-phellandrine, and an unknown compound. In contrast, attacked trees showed significantly higher percentage of camphene and another unknown terpene component. Of the monoterpenes that have been tested, camphene is considered to be one of the least toxic to SPB. Identification of the two unknown monoterpenes is ongoing. The results suggest that terpene profiles may be a useful tool for identifying susceptible and "resistant" genotypes and could be used in tree improvement programs for selection of families with less susceptibility to SPB attack.

Keywords: *Pinus taeda* L., *Dendroctonus frontalis* Zimm., terpene.

SCOTS PINE (*Pinus sylvestris* L.) BREEDING SRATEGIES IN SPAIN

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Abstract. Scots pine covers the largest natural area of the genus *Pinus*, from 8 W to 141 E degrees of longitude and from 37 S to 70 N degrees of latitude. In Spain it reaches the southern limit of the species, on the main mountain ranges of the country and living in many different ecological conditions. It occupies around one million of hectares and half of them came from artificial afforestation.

Adopted strategies are extensive because the obtained propagation material (seed) will be used in a great variety of ecological conditions in time (long rotation) and space (high site variability).

The first step to plan a improvement program for the species was to define the provenance regions as a set of territories with high ecological and genetic homogenity. So 17 provenance regions were established based on taxonomic, climatic and pedologic previous information. These regions were afterwards identified as breeding zones for operative proposes.

Adaptation, growth and gene conservation are the main objectives of the program. Depending of the provenance region, the importance of each of these three objectives is different.

Genetic variability studies allow us to select a set of populations to be used in a wide range of conditions. The results indicate the non significance of genotype-environment interaction for growth and phenology. It can be assumed Spanish provenances have a lower vigor than mid-european ones, but they are best adapted to extreme drought conditions and to typically Mediterranean erratic fluctuations of climate. It seems that Spanish provenances have a more "conservative" growth startegy.

On the strength of phytoclimatic similarity, importance of the provenance and seed demand, all the provenances have been classified into three categories with different strategies adopted. In the most demanded provenances, seed orchards and seed stands have been conducted. Some seed orchards have been monitored from their begining till nowadays. The provenances which have only local interest or whose seed demand is low, only seed stands selections have bee4n conducted. Up to date 20 seed stands have been selected from 4 provenance regions and 4 seed orchards (11,8 ha) from 3 provenance regions have been established. All of them (stands and orchards) make the National Catalogue of Forestry Reproduction Material for the species.

Finally there are a subset of preservation populations were protection and regeneration measures have to be adopted in order to preservce their genetic resources for future generations.

Keywords: *Pinus sylvestris*, breeding strategies, provenance trial, seed orchard, variability.

IDENTIFICATION OF QUANTITATIVE TRAIT LOCI AFFECTING ROOTING IN LOBLOLLY PINE

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Abstract. Genetic control of rooting and root development is poorly-understood in conifers. This study was designed to identify quantitative trait loci (QTLs) affecting rooting of loblolly pine (*Pinus taeda* L.) stem cuttings. In each of four seasons (winter 1995, summer 1995, winter 1996 and summer 1996), sixteen cuttings were taken from each of 384 open-pollinated offspring of loblolly pine clone 9-1020, which were being maintained as hedges. Cuttings were rooted in greenhouse environments, and the rooting percentage and average number of new roots were recorded for each hedge. Megagametophyte DNA from each of the 384 offspring was scored for 73 RAPD markers.

Probable QTLs were detected at various significance levels on six linkage groups, each of which affected both rooting percentage and number of roots. Three of the QTLs affected rooting in both the winter and summer seasons, while two had effects only on winter rooting and one only on summer rooting. Individual QTLs explained estimated differences of 3 to 9 percent in rooting success and 0.1 to 0.2 roots per cutting. The occurrence of candidate QTLs with multiple tests and traits, and the consistency with which positive or negative effects were associated with particular marker combinations, are strongly suggestive of real effects. The detection of season-specific QTLs suggests the interaction of modifying developmental pathways with the root initiation genetic program. This information may help provide insight into the function of candidate genes.

Keywords: QTL mapping, rooting, loblolly pine, *Pinus taeda* L.

GROWTH OF TISSUE CULTURE DERIVED SWEETGUM IN THE FIELD - FIRST FIFTEEN YEARS

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Abstract. In 1981 a block of 96 sweetgum plantlets was planted on a sloping site at the University of Georgia's Whitehall Forest near Athens. Periodically, measurements were made of height and root collar diameter or DBH. Growth appeared to be highly dependent on the position on the slope where the plantlets were planted. These plantlets were obtained using a tissue culture technique with a low rate of multiplication. Multiplication rates were increased and about 800 plantlets were grown for one season in a nursery bed. Since that time, we have been able to root the adventitious shoots directly in potting mix. It is now potentially time to set up a larger trial.

Keywords: *Liquidambar styraciflua,* adventitious shoots, field test

EFFECTS OF PROPAGATION MEDIA ON THE ROOTING OF LOBLOLLY PINE

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Abstract. Four types of propagation media were evaluated for their effects on the rooting of loblolly pine cuttings: (1) a 1:2 (v:v) mixture of peat:perlite in 10-in^3 Ray Leach Super Cells™ (SC), (2) the same peat:perlite mixture in 20x10x2-in (LxWxH) propagation flats (PF), (3) Oasis Rootcubes® (RC), and (4) Oasis Wedges® (W). Cutting material originated from the three best-rooting clones and three worst-rooting clones in each of two related full-sib families. The cuttings were 28-months-old (from seed) at the time they were set on May 25, 1996. Five cuttings were set for each media type/family/rooting class/clone combination in each of three blocks. All effects were considered fixed and clones were nested within family/rooting class. Intermittent mist (8 sec/15 min) was provided by stationary Ray-Jet® nozzles with a delivery rate of 7 gpm. After 90 days, the cuttings were evaluated for presence of roots, and number and average length of those roots longer than ½-in. Rooting percentage data revealed significant effects for media type, rooting class, and clone. Mean rooting percentages were 51, 31, 30, and 26% for SC, PF, W, and RC, respectively. Rooted cuttings in the SC medium had significantly more and longer roots compared to rooted cuttings in any of the other three media. In addition, there were fewer cases of "L-shaped" roots with rooted cuttings grown in the SC medium. Lateral root development was greater in the peat:perlite media compared to the Oasis material. All of this probably is a function of better drainage in the peat:perlite and the Super Cell containers training the developing roots in a downward direction. The intermittent mist simply delivered too much water to the Oasis material. This rooting trial is currently being repeated under a traveling mist boom system. Early results indicate that while rooting percentages have increased in the peat:perlite medium, the Oasis medium became too dry during the critical early stages of root initiation. More research apparently is needed if Oasis material is to be used in propagating difficult-to-root species such as loblolly pine.

Keywords: *Pinus taeda* L., vegetative propagation, rooted cuttings.

HYBRID LARCH - SOUTHERN PINE OF THE NORTH ?

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Abstract. In Quebec, the fastest growing conifer used for reforestation is *Larix* x *eurolepis* Henry, a hybrid between two exotic species : european larch (*L. decidua* Mill.) and japanese larch (*L. kaempferi* [Lamb.] Carr.). Both species have restricted natural ranges in mountainous areas of central Europe and on the island of Honshu (Japan). Distinctive traits exist between the parental species ; hybrids show intermediate characters, but they are not always easy to identify. Larches are shade intolerant and require to be kept free of competition for a few years after plantation. Hybrid larch grows best on a deep (> 1 m), moist loam that is well to moderately well drained. In Quebec, suitable areas for hybrid larch cover the hardwoods and the mixed woods forest zones (most of southern Quebec).

An international experiment was started in 1973, in the Great Lakes - St.Lawrence forest region, to assess the growth rate and form of larch hybrids from the best and most highly selected material available in northern Europe at this time. In Quebec, 20 seedlots (6 seed orchards, 14 half-sib progenies) were tested at eight locations with 13 seedlots of european larch and 10 of japanese larch as controls. The seedlings were planted in 1977 and 1978, at a spacing of 2.5 m x 2.5 m. Results from the three most comprehensive tests are presented to compare the growth of five hybrid seed orchard seedlots with that of the controls (five european and eight japanese).

Fifteen years after plantation (trees aged 19 years), the average "dominant" height of the trees is 12.0 m for the hybrids, compared to 10.2 m for the controls. The best hybrid source (a Danish seed orchard : F.H. 211, SORØ1) has a "dominant" height of 12.8 m. Dominant height is here defined as the average height of the trees above the median. Based on growth models developped in Quebec for european and japanese larch plantations established on unprepared forest sites with unimproved seedlings and a 60 % survival rate, at a spacing of 2 m x 2 m (Bolghari and Bertrand 1984), the expected productivity of the hybrids is 245 m^3/ha (total volume) at 35 years (total age), compared to 188 m^3/ha for the controls. For the best hybrid source, the volume per hectare at 35 years is 270 m^3. Based on mean annual increments, hybrid larch has a growth potential equivalent to that of slash pine and loblolly pine in standard plantations of average site index (Lundgren 1982).

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Lundgren, A.P., 1982. Can red pine in the Lake States outproduce loblolly and slash pine in the south ? p. 337-344 in Proc., Artificial Regeneration of Conifers in the Upper Great Lakes Region. Oct. 26-28, 1982, Green Bay, Wisc.

ENHANCED DETECTION OF GENETIC EFFECTS FROM FORESTRY FIELD TESTS USING SPATIAL ANALYSIS

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Abstract. The precision of forest genetic field tests is limited by heterogeneous environmental variation within blocks caused by gradients in moisture, nutrients, or other factors that vary over a scale smaller than plot size. This nonrandom variation reduces heritability and obscures genetic differences. Here, we use spatial analysis to remove environmental trends from field data and increased the statistical power to detect genetic effects. We applied a random field model to height data that was defined by position (row and column) in a field test. This model described broad environmental trends for a whole (10 x 10) tree family block plot. Residuals from the random field model contain "detrended" phenotypic information (systematic environmental variation removed) and decrease the intensity of autocorrelation between neighboring individuals.

A field test established in Lumberton, North Carolina, with the objective to detect average effects QTLs for shoot elongation in loblolly pine was used as an example. Five 100-tree rectangular family plots in blocks 4, 5, 6, 8 and 9 were evaluated for second year height increment. From the overall data (500 trees), a subsample of individuals was taken to obtain RAPD marker information from megagametophytes.

Results show that the random field method is efficient in removing fixed environmental trends in the data which due to their intensity usually mask real genetic differences. Considering the data from forest genetics field experiments within a spatial framework is more natural and can refine statistical analysis and improve the reliability of the inferences drawn from it. Taking into account spatial relationships lowers both environmental and phenotypic variances and increases heritability.

Keywords. Data analysis, spatial statistics, spatial autocorrelation, QTL, heritability.

LOBLOLLY PINE PROVENANCE AND FAMILY DIFFERENCES IN WATER USE IN RESPONSE TO ATMOSPHERIC CO2 ENRICHMENT

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Abstract. Elevated CO_2 concentrations have been reported to reduce stomatal conductance and water use in tree species. To determine whether there are family or provenance differences in loblolly pine (*Pinus taeda* L.) water use in response to short-term increases in atmospheric CO_2 concentration, whole plant water use of seedlings derived from 10 Interior families and 13 Coastal Plain families was measured at ambient and twice ambient CO_2 concentrations. Whole plant water use was measured a second time, four months later, to test whether the results changed with increasing size or age. When measured the first time, CO_2 enrichment decreased whole plant water use of the Coastal Plain seedlings by 4.7% which was significantly greater than the 2.5% decrease in water use of seedlings from the Interior region. Family within region differences in the percent decrease in water use due to CO_2 enrichment were not significant. Measured the second time, CO_2 enrichment decreased whole plant water use of Coastal Plain seedlings by 18.1% which was significantly greater than the 15.1% decrease of the Interior seedlings. During this second sampling, some of the family differences within region were significant. Size and growth rates of seedlings from different regions were significantly different but were not correlated with the percent decrease in water use due to CO_2 enrichment.

Key words: *Pinus taeda* L., half-sib families, provenance, whole plant water use

USDA FOREST SERVICE, FOREST HEALTH PROTECTION RESISTANCE SCREENING CENTER DISPLAY

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Abstract. The Resistance Screening Center (RSC) is operated by the Forest Health Protection unit of the USDA Forest Service, Southern Region, State and Private Forestry. The Center is located at the Bent Creek Experimental Forest near Asheville, NC. The Center evaluates seedlings for resistance to disease, primarily fusiform rust (caused by Cronartium quercuum F. sp. fusiforme) and pitch canker (caused by Fusarium moniliforme var. subglutinans) as a service to tree improvement specialists, seed orchard managers, scientists, government agencies, research institutions, universities, and private industry. Testing enables clients to obtain information on the relative resistance of their materials in much less time than is possible in field progeny tests. The RSC has the flexibility to modify the current screening procedures to accommodate specialized requests. This allows researchers to use the RSC as an additional experimental tool. By using information from Resistance Screening Center tests, trees producing resistant progeny can be identified or questions may be answered concerning such things as the nature of variation in the rust fungus or the effectiveness of fungicides.

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