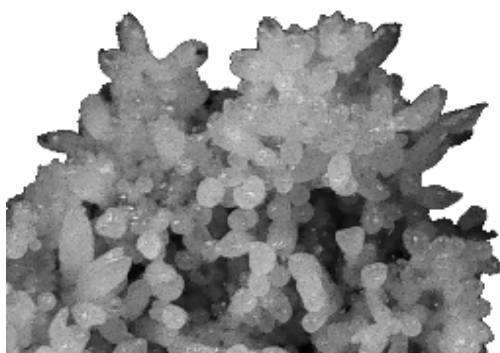


Dendrome

Forest Tree Genome Research Updates

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Cluster of sweetgum (*Liquidambar styraciflua*) somatic embryos

Photographer: Scott Merkle and P.J. Battle

Forest Biotechnology Research at the University of Georgia

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The School of Forest Resources at the University of Georgia has a long tradition of conducting research in the area now known as “forest biotechnology.” In fact, scientists at the School were working with cell and tissue cultures of forest trees decades before “biotechnology” was part of the English language. Dr. Claud Brown (now Professor Emeritus) joined the faculty of the School in the 1960s and conducted ground-breaking research using *in vitro* techniques, for example, to demonstrate the role of pressure in the differentiation of xylem and phloem tissues from cambial initials. The first defined medium for growth of pine callus, Brown and Lawrence medium, was developed in Brown’s laboratory in 1968. In 1975, Dr. Harry Sommer, who remains on the faculty today (see below), working with Brown and Dr. Paul Kormanick, was the first to demonstrate *in vitro* regeneration of a pine species via adventitious buds.

Today, the school has not only maintained its efforts in cell and tissue culture of forest trees, but expanded into such areas as molecular genetics of trees and their pathogens and genetic engineering of metabolic pathways in trees. The number of faculty involved in this area of research has grown to four and a new position in tree molecular genetics/genomics will be filled in the near future. We would like to take this opportunity to introduce our research programs and to present a snapshot of our progress in the field.

Dr. Harry Sommer originally came to the School in 1972 to join Dr. Claud Brown’s lab as a postdoctoral associate. He returned to join the School’s faculty in 1975. Sommer and Brown’s early successes in obtaining adventitious buds in conifers led to a series of studies by graduate students and visiting scientists, who applied the technique to different pine species and hybrids. In addition, the technique was picked up readily by many other labs interested in *in vitro* propagation of conifers. A collaboration was started with Dr. Hazel Wetzstein (UGA Horticulture Department) to investigate the potential for propagation of sweetgum via adventitious buds. By using liquid suspension, Sommer and Wetzstein were able to obtain high rates of shoot production. At present, some of the trees derived from these cultures have been in the field for 16 years. Sommer and Wetzstein

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also used electron microscopy to investigate the changes that take place during hardening off of the sweetgum plantlets. Recently, Sommer has returned to the study of organogenesis in conifers, with the goal of determining which phenocritical events control the development of adventitious buds *in vitro*. Sommer has also devoted considerable effort over the past decade to research on somatic embryogenesis of selected conifers. He was able to initiate embryogenic cultures of Douglas-fir, but development of the embryos beyond the precotyledonary stage was problematic.

Scott Merkle came to the School in 1984 to work as a postdoctoral associate with Drs. Brown and Sommer, and subsequently joined the faculty in 1987. Since he has been at UGA, he has focused on developing embryogenic regeneration systems for a number of hardwood species and on applying the embryogenic cultures for mass propagation and gene transfer research. Species for which embryogenic cultures have been developed in the Merkle lab include yellow-poplar, hybrid *Liriodendron*, black locust, American chestnut, various magnolias, and sweetgum. In 1992, Dr. Dayton Wilde (currently at Westvaco Corp.), who worked as a postdoc with Merkle and Dr. Richard Meagher of the UGA Genetics Department, used biolistics to transform embryogenic yellow-poplar cultures and regenerate plants transformed with marker genes. Some of these transformed trees remain in the greenhouse (although they had to be cut back more than once) where their tissues continue to express GUS and NPTII over 5 years later. Approximately 20 of the transformed trees will be moved to a field trial site this winter.

The development of a reliable transformation system for yellow-poplar opened up great possibilities for testing different genes in these trees, including genes for heavy metal resistance, lignin modulation and early flowering. Work with lignin modification and early flowering will be covered in Dr. Dean's section (see below). With regard to heavy metal resistance and detoxification, Dr. Clayton Rugh, working with Merkle and Meagher, used biolistics to generate yellow-poplar trees that expressed a modified bacterial mercuric ion reductase (*merA*) gene. The gene was cloned in Dr. Anne Summers' (UGA Genetics Department) lab, and in Meagher's lab, its sequence was modified using overlap extension PCR to optimize its expression in plants. Rugh showed that yellow-poplar trees transformed with *merA* could survive and grow on levels of mercuric ion that were normally toxic to control trees. Furthermore, transformed trees growing on medium containing mercuric ion generated metallic mercury vapor at up to ten times the rate of control trees, indicating that the reductase gene was expressed and functional. The long-term goal of the research is to provide plants that can be used for remediation of sites contaminated with mercury and other heavy metals.

The most recently-developed embryogenic culture system in the Merkle lab is one for sweetgum. Most embryogenic systems for forest trees reported to date have used seed or

seedling tissues as explants. Thus, the plants derived from such cultures are of unknown genetic value. In the case of sweetgum, however, staminate inflorescence tissues from mature trees cultured on medium supplemented with thiodiazuron proved to be amenable to initiation of embryogenic cultures. While protocols for the germination and conversion of the somatic embryos remain to be optimized, the use of inflorescence tissues as explants opens the potential to apply somatic embryogenesis to clone elite genotypes of this species, which is the object of increasing interest as a plantation species in the southeastern U.S..

Sarah Covert joined the School in 1993 as an Assistant Professor. Her laboratory currently employs a mixture of molecular and genetic techniques to study three different fungal pathogens of plants. Their long term goal is to gather basic information about the host-pathogen interactions and then to use this knowledge to formulate novel disease control methods, perhaps via genetic engineering of the host plants.

Two of the pathogens studied in the Covert lab affect pine species of commercial importance in the southeastern United States.
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Dendrome

Dendrome is a bi-annual research update on forest tree genomes. If you would like to submit an article or announcement, please do so by July 15, 1998. Please include your name, address, fax number or email address. Pictures are welcomed.

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States. The first of these is *Cronartium quercuum* f. sp. *fusiforme* (*Cqf*), the causal agent of fusiform rust. This disease is characterized by the formation of galls on the woody tissue of infected pines and causes significant economic losses due to mortality and reduced wood quality. The major fusiform rust project in the Covert lab focuses on determining how *Cqf* causes gall formation on pine. They are using a PCR-based technique known as differential display for this purpose. To date, several cDNAs that are differentially transcribed in galled and healthy pine tissue have been isolated. Current and future work will include expanding this collection of clones, determining their genome of origin, and assessing the role of all clones in gall formation. Because they might be useful reagents for the development of resistant trees, cloning the promoters from pine genes induced by *Cqf* infection is also a priority.

Dr. Covert's lab is also studying *Fusarium subglutinans* f. sp. *pini* (*Fsp*), the causal agent of pitch canker disease. This disease is characterized by the formation of resinous cankers which cause shoot dieback on infected pines. The native population of *Pinus radiata* in California is currently under heavy attack by this disease, and it has also been of economic significance in parts of the southeastern US. Recently, work in the Covert lab has focused on determining how to induce *Fsp* to reproduce sexually in the laboratory, and on a molecular characterization of mating type loci in this fungus. They have found that *Fsp* requires a different temperature for mating than other, closely related *Fusarium* species and have cloned one of the *Fsp* mating type genes. The methods they have developed will allow them to conduct a genetic analysis of pathogenicity in *Fsp* in the future.

In 1990, **Jeffrey Dean** was recruited from the USDA Plant Hormone Laboratory (Beltsville, MD) to join Dr. Karl-Erik Eriksson's research group in the UGA Department of Biochemistry and Molecular Biology as an Associate Research Scientist. While a member of the Eriksson laboratory, Dean was heavily involved with a number of projects that sought biotechnological answers to problems in the pulp and paper industry. Amongst these projects were efforts to clone and produce thermostable xylanases for use in chlorine-free pulp bleaching processes, studies of the extracellular enzymes produced by white-rot fungi for the breakdown of lignocellulosic materials, and the development of new molecular models to describe the interactions between lignin and elements in the surrounding cell wall matrix. At this time, Dean also initiated a research program focused on identifying factors that control lignin deposition during xylogenesis, particularly with respect to the oxidative enzymes that bring about monolignol polymerization within the wall matrix. In the course of these studies, Dean and co-workers (Drs. Lan Liu and Raja Sterjiades) were able to demonstrate a tight

correlation between lignification and the expression of a blue copper oxidase commonly referred to as laccase. They also showed that the purified enzyme could catalyze monolignol polymerization *in vitro*. Dr. Peter LaFayette, working with Dean, subsequently cloned and characterized several laccase genes, and in collaboration with Dr. Merkle, a series of constructs designed to test laccase gene expression and function have been used to transform yellow-poplar. One of the most interesting phenotypes seen to date comes from the constitutive expression of a laccase gene cloned from sycamore maple. Yellow-poplar trees expressing this gene grow very slowly and their tissues contain elevated levels of phenolic materials which appear to be localized primarily in their cell walls.

Dr. Dean moved to the School of Forest Resources as an Assistant Professor in May of 1996, and he and his research group continue to study the regulation of laccase gene expression. Their studies of laccase structure-function relationships have been facilitated by Dean's affiliations with the Center for Metalloenzyme Studies and the Complex Carbohydrate Research Center, both of which are located on the UGA campus.

Most recently, a collaborative project between Jeff Dean, Scott Merkle and Sarah Covert at UGA, as well as Drs. John Cairney and Jerry Pullman at the Institute for Paper Science and Technology in Atlanta, has focused on developing a better understanding of the genes that regulate flower and strobili development in trees. The intent is to develop genetic constructs that could be manipulated in order to stimulate trees to flower in a controlled manner at a juvenile stage, or alternatively could be used to prevent the development of reproductive structures altogether. Although this project is at a relatively youthful stage, a number of transgenic trees carrying genes to stimulate flowering are already in the greenhouse, and trees carrying sterility constructs are very close behind. Concurrently, work is underway at UGA to isolate sweetgum and yellow-poplar homologs of genes that regulate floral morphogenesis, such as *Leafy* and *Apetala*.

Currently, the School is in the process of recruiting a new assistant professor in the area of molecular genetics/genomics. The intention is that the new scientist will conduct research resulting in the mapping, identification and cloning of genes controlling growth rates, as well as pest and pathogen resistance, in southern forest species. In addition to collaborating with faculty in the School of Forest Resources in order to improve forest productivity in the southern U.S., this new faculty member will likely play a significant role in the new Agricultural Genomics Initiative that is underway at the University of Georgia.



Tree Improvement: Its Contribution To Sustainable Development

26th Meeting of the Canadian Tree Improvement Association

Jean Beaulieu ¹, M. Villeneuve ², and Jean Bousquet ³

The Canadian Tree Improvement Association (CTIA) held its 26th biennial meeting at Université Laval, Sainte-Foy, near Québec City, Québec (Canada) August 18-21, 1997. The meeting was co-sponsored by the Canadian Forest Service, the Quebec Department of Natural Resources, Université Laval and Forintek Canada Corp.

The conference began with a one-day symposium on tree seed research for some attendees, while others participated in a workshop on wood quality and end-product value. The latter was jointly organized by the CTIA and IUFRO. Three keynote speakers were invited by the Tree Seed Working Group to address various topics related to artificial pollination in seed orchards. Hence, Marc Bonnet-Masimbert (France) gave an interesting talk on artificial pollination in larch and seed orchard management, Floyd Bridgwater (USA) gave a demonstration of the effectiveness of controlled mass pollination for increasing genetic gains in loblolly pine, and Greg Adams (Canada) explained the potential for using controlled parentage in operational reforestation.

The first keynote speaker of the joint CTIA-IUFRO workshop on wood quality was Bruce Zobel (USA). He talked about the genetics of wood and the importance of having sample sizes large enough to obtain accurate estimates of wood properties, as the genetic variation in these traits is very large. He also suggested considering wood density as a secondary trait for selection. The priority should be growth traits. Hans van Buijtenen (USA) gave a talk on the potential of marker-aided selection (MAS) for wood quality. He used both deterministic and stochastic models to study the effectiveness of MAS in an actual breeding program. His results suggest that selection based only on markers would be very attractive if the trait to be selected is very expensive to measure. For traits that are not, the best method would be marker-aided selection, followed by direct selection. The principles for the development and use of nondestructive methods for the evaluation of wood quality in research and testing were presented by Ladislav Kucera (Switzerland). He also gave examples of the state of the art in field applications and the methods used to obtain the required estimates. Finally, Charles Sorenson (New Zealand) presented the radiata pine case study as an example of the significance of wood quality in tree breeding. This one-day joint workshop ended with voluntary speakers who addressed questions such as breeding objectives for pulp of eucalypts, improvement of western hemlock pulp, paper and structural wood quality, investigation of wood quality through molecular markers in larch, and many other subjects.

The first plenary session on the second day was devoted to current knowledge in tree breeding. First, Jean Beaulieu (Canada) gave an overview of progress in
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tree breeding in Québec with emphasis on white spruce and black spruce. After the breeding strategies used were presented, results were reported. The second-generation breeding cycle is well under way and expected genetic gains for growth traits exceed 20%. Breeders have capitalized on various techniques ranging from traditional breeding to molecular breeding in order to improve growth and wood traits in these spruce species. Steve McKeand (USA) reported results on genetic gains after two generations of tree improvement in loblolly pine in the southeastern United States. He showed that the estimated gains for height from second-generation seed orchards ranged from 6% to 10% over unimproved checklots in different regions and that the gains in volume at rotation age were substantially higher. Moreover, the best families were 10% to 20% above the first-generation seed orchard mix in height growth, indicating that progress is still continuing at a good pace. A substantial number of second-generation selections also have less rust infection and better average bole straightness than unimproved checklots.

The third keynote speaker, Bill Libby (USA), presented his thoughts on the problem of genetic diversity in clonal plantations and recommendations for lowering the risks of unacceptable loss. He first discussed the concept of diversity, with emphasis on the importance of adaptive diversity and clonal deployment strategies to maximize production and diversity. He suggested that Mosaic Of Monoclonal Stands (MOMS) may develop richer ecosystems than Wide-spread Intimately Mixed Plantations (WIMPs) because there is less ecological space for associated plant species in the developing WIMP ecosystem. However, this could be different for pests, pathogens and other organisms that live on trees. He then discussed the importance of measuring the genetic diversity at the within-provenance or

breeding program level. He presented three ways to measure and monitor genetic diversity, i.e. the effective number (N_e), the status number (N_s) and the random-pair identity (N_{ri}). He suggested that the deployed number of unrelated clones should be about 20. With such a number, one is not likely to lose adaptive variation in the population. Furthermore, three variables should be considered when measuring and monitoring deployed genetic diversity, i.e. the census number, relatedness, and proportional deployment. Finally, he recommended maintaining and deploying amounts and structures of genetic diversity such that: a) clonal plantations will have a lower risk of unacceptable loss than do seedling plantations, and b) clonal plantations will have a lower risk of unacceptable loss than do natural stands. This would proactively blunt probable criticism.

Alvin Yanchuk (Canada) gave an interesting talk on the elaboration of low-risk strategies for the development of resistance to forest pests. He first reviewed the various types of resistance mechanisms that exist and the terminology used. Then, he discussed three genetic issues that underlie the basis of developing and deploying resistant material in forestry, i.e., a) genetic uniformity, b) the target sets of genes, and c) genetic correlations between resistance and other traits. After talking briefly about the breeding options, he set out deployment strategies for improved pest-resistant material and used, as an example, the breeding program for resistance of spruce to white pine weevil in British Columbia. The last keynote speaker for that session was Dave Neale (USA). He gave a stimulating talk on recent progress towards Quantitative Trait Loci (QTL) detection and verification for wood quality traits in loblolly pine and adaptive traits in Douglas-fir. Populations of modest size (~ 200 progeny of single three-generation pedigrees) have been used so far for

QTL mapping. He reported the detection of five QTLs for wood specific gravity in loblolly pine. The use of complete radial cores as well as X-ray densitometry scans showed that the effect of wood specific gravity QTLs varies from ring to ring and from early wood to late wood. QTLs are also sought for wood chemistry components such as lignin, cellulose, and hemicellulose. A validation experiment has also been initiated for these QTLs using 500 new progeny outplanted in a highly uniform test plantation. Neale's work on Douglas-fir is for QTLs for bud flush and spring and fall cold hardiness. So far four QTLs for bud flush have been detected and validation has been initiated. Furthermore, a clonally replicated mapping population of 500 new progeny could make it possible to identify QTLs responding to different environmental stimuli for bud flush and bud set. Hence, marker-aided breeding will be possible in the near future.

This plenary session was followed by talks from voluntary speakers that dealt with biotechnology and molecular population genetics. Various topics were presented. Ron Schmidting (USA), the first speaker, talked about restoration of longleaf pine in the southeastern United States, while Raj Ahuja (Germany) closed the session with a presentation on the paradox of genetic engineering in trees, discussing concerns about how to direct the integration of alien genes and long-term stability. This talk sparked the discussion in an evening workshop on the intellectual property of genetically improved material and on issues related to transgenic plants.

The meeting continued on the third morning with a plenary session bringing together four speakers invited to give their points of view on how breeding programs can best contribute to sustainable development. The four Canadian speakers were Gilles Vallée (tree

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improvement), Martin Lechowicz (ecology), Jean-Claude Mercier (socioeconomics) and Greg Adams (forest industry). While these specialists all had different backgrounds and in some cases experience not directly related to tree breeding, they came out with a strong consensus on the importance of tree breeding for meeting the demand for wood products and protecting a larger portion of natural ecosystems, and they agreed that this should be better conveyed by tree breeders to the general public and policy makers. In the afternoon, two sessions with voluntary speakers on physiological genetics and pest resistance and on field testing and genetic gains took place. For some, the meeting finished with these talks. Others, on the last day, participated in a post-conference tour.

This meeting succeeded in bringing together specialists from various fields such as forest genetics, tree breeding, molecular biology, wood sciences, ecology and economics. Discussions were very fruitful and a consensus emerged on the valuable contribution of tree improvement to sustainable forest management and on the need to better target breeding programs towards end-product values.

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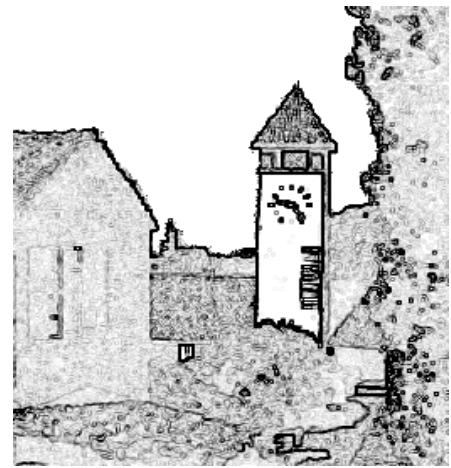
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Report on 1997 Meetings of the Western Forest Genetics Association (WFGA)

Tom Conkle (tconkle/psw@fs.fed.us)

The Institute of Forest Genetics hosted the 1997 WFGA meetings in Berkeley on the University of California's Clark Kerr Campus from the 4th to the 7th of August. Twin themes were molecular forestry and biodiversity.



Molecular forestry was highlighted during a day-long educational symposium hosted by David Neale. Speakers Craig Echt, Mitch Sewell, Zeki Kaya, Claire Kinlaw, John Carlson and Toby Bradshaw presented highly informative talks on molecular markers, linkage maps, gene discovery, quantitative trait loci, genome organization and molecular phylogenies in forest trees. All the subjects were "hot topics" with presenters discussing basic elements of the sciences and current progress in research on tree species, both hardwoods and conifers. About 50 western forest geneticists participated in the symposium, that is memorable for the high quality of talks and the friendly atmosphere of the CAL Faculty Club.

California biodiversity was highlighted during a mini-symposium the morning following the molecular forestry symposium. Connie Millar, fresh from three years of leadership of a Congressionally sponsored science team on Sierra Nevada Ecosystems, hosted talks on forest ecology. Speakers Deb Rogers, Dave Graber, Carl Skinner and Dave Stoms spoke on the condition of plants, animals and forest processes and outlined plan options for Sierran forest ecosystems. Presentations had obvious relevance to WFGA's genetic research talks that followed.

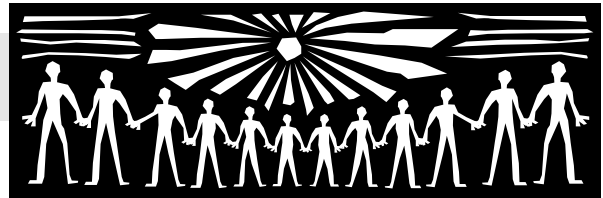
About 20 contributed talks were presented to 75 members in attendance. Speakers reported significant advances in mapping genes, assessing molecular aspects of populations, and analyzing growth responses in laboratory and field trials. Graduate student contributions were excellent, Greg O'Neill's presentation from research on the genetics of fall cold hardiness in coastal Douglas-fir, done at Oregon State University, won the Association's 1997 Critchfield Scholarship; congratulations Greg!

A field trip to Placerville highlighted Bro Kinloch's research on sugar pine resistance to blister rust, Dave Neale's molecular work tagging sugar pines's major resistance gene, and Safiya Samman's Region 5 program to screen native sugar pines for resistance. Members participated in a poster session, observed research underway at the Institute of Forest Genetics and visited the Forest Services's National Gel Electrophoresis Laboratory at the Placerville Nursery.

Thanks go to Richard Snieszko for serving as WFGA Chair for 1997. Brad St. Clair became Chair for 1998, Lauren Fins serves as Vice-Chair, John Russell is newly elected to Secretary and Treasurer.

The 1998 WFGA Meetings will be held in British Columbia on the University of Victoria Campus from the 21st to the 26th of June. WFGA combines with the North American Forest Biology group in a joint meeting on Frontiers of Forest Biology. Complete 1998 Conference information is available at <http://www.uvcs.uvic.ca/conference/NAFBWFGA/>.

Calendar of Meetings



7-11 June, 1998, Eighth Meeting of the Conifer Biotechnology Working Group, New Brunswick, New Jersey, USA.

The program will focus on recent developments in conifer biotechnology, including genetic transformation, somatic embryogenesis, molecular biology, biochemistry, molecular genetics, genome studies, micropropagation, clonal forestry, and developmental biology. The conference will feature plenary sessions with invited speakers, contributed oral presentations, and poster sessions. In addition, workshops on selected topics will be organized. Contact Dr. Steve Wann, 8th CBWG Conference, Union Camp Corporation, P.O. Box 3301, Princeton, New Jersey 08543-3301 USA for second announcement.

8-12 June, 1998, International Symposium on Advanced Technology in Environment and Natural Resources, Rovaniemi, Finland. For more information check out links: <http://www.metla.fi/event/rt98> for information and <http://metla.fi/event/rt98/registration> to register.

21-26 June, 1998, North American Forest Biology Workshop and Western Forest Genetics Meeting, Victoria, British Columbia, Canada and on June 25 the North American Quantitative Forest Genetics Group Meeting theme is Frontiers of Forest Biology, This joint meeting will provide interdisciplinary discussions of forest biology frontiers including Genetics, Silviculture, Plant Physiology, Ecology and Conservation. Abstract deadline is December 15, 1997. If interested in attending, contact NAFBQFGA@uvcs.uvic.ca or check out link <http://www.uvcs.uvic.ca/conferce/NAFBWFGA/>.

16-18 August, 1998, 18th International Congress of Genetics, Kunming, Yunnan Province, People's Republic of China. This conference will contain oral and poster presentations on Genetics and Conservation of Biodiversity. Three symposia on population genetics, evolutionary genetics, conservation genetics and other related subjects. The deadline for paper abstracts is May 31st, 1998. Visit homepage <http://www.hgc.sph.uth.tmc.edu/others/kunming/>.

22-28 August, 1998, Contributions of genetics to the sustained management of global forest resources, Beijing, China. IUFRO Division 2 Conference. The Conference is intended as a consultative meeting of restricted size. Registration fee will be 400 USD. Participants should send preliminary registration forms to: the Scientific Organization Committee secretariat in Sopron, Hungary. More information available at <http://iufro.boku.ac.at/iufro/test/prof.htm>.

14-18, September, 1998, International Poplar Symposium II, Orleans, France. This five day meeting will blend invited lectures, presentations and posters, with a two day field trip to research sites and poplar cultivation along the Loire River. Contact: Marc Villar, IPS II, INRA, Station d' Amelioration des Arbes Forestiers, F- 45160 Ardon, France or email: villar@orleans.inra.fr

1-3 October, 1998, International Elm Conference, Lisle, Illinois, USA. This conference will focus on contributed papers on Elm Research such as Dutch elm disease and other elm problems. For further information, contact: Christopher P. Dunn, Director of Research, The Morton Arboretum, 4100 Illinois Route 53, Lisle, Illinois 60532, phone 630-719-2423, email : cdunn@mortonarb.org or browse <http://www.mortonarb.org>.

12-15 October, 1998, IUFRO Seed Symposium on Recalcitrant Seeds, Kuala Lumpur, Malaysia. In conjunction with this symposium, a Cryopreservation workshop will also be organized 7-9th October. For more information please contact: Dr. Daniel Baskaran Krishnapillay, Forest Research Institute Malaysia (FRIM), Kepong, 52109 Kuala Lumpur, Malaysia. Email : baskaran@frim.gov.my, fax: 603-6367753, 603-6342758.

12-17 October, 1998, Forest Ecosystem and Land Use in Mountain Areas IUFRO Division 4 Conference, Seoul, Korea. Republic of Korea. Homepages <http://plaza.snu.ac.kr/~leedk/> and <http://aginfo.snu.ac.kr/~leedk>. For further information contact Don K. Lee at leedk@plaza.snu.ac.kr.

Genetics of Radiata Pine
1-4 December 1997
Rotorua, New Zealand



IUFRO Working Party S2.02.19

Peter Kanowski
ANU Forestry, Canberra

Despite the commercial and genetic importance of *Pinus radiata* D. Don, December's meeting was only the second of the IUFRO Working Party devoted to its genetics; the first, 17 years previously, had also been hosted in Rotorua by NZ Forest Research Institute. Whilst radiata pine geneticists and breeders have been prominent in many other fora in the intervening period, this Working Party meeting provided an opportunity for both focus and reflection amongst the spa baths, steaming geysers and radiata plantations of Rotorua.

The majority of the 120 participants were based in New Zealand or Australia, with strong presence also from Chile and North America, and representation from South Africa and Europe. Most of those who had been prominent at the 1982 meeting were present in 1997, attesting both to their longevity and the enduring appeal of radiata pine; a quarter of the 45 authors contributing papers to the 1997 meeting had also written in 1982. The two largest disciplinary groups, present in about equal numbers, were the quantitative and molecular geneticists; both were equally assertive, but the latter generally more impassioned.

The genetic and economic contexts of the topic were set in keynote papers by Oliver Mayo and Peter Olsen, respectively. Subsequent sessions addressed evolutionary history, taxonomy and genomic structure; inter- and intra-provenance variation and genetic resources; reproductive biology; breeding and deployment goals and strategies; and the integration of quantitative and molecular genetics. The four conference days were followed by a one-day gene mapping workshop. Papers from the conference and abstracts from the workshop are available as NZFRI Bulletin 203, commendably edited by Rowland Burdon and Judith Moore.

Bill Libby, who spoke at this meeting about the origins of radiata pine, had presented the concluding synthesis to the 1982 meeting. He noted then that, as one of the few domesticated tree species, radiata pine "will rank among tree species at a level of importance similar to that

of wheat, rice and corn among agronomic species". In attempting to fill the same role in 1997 as Bill had 15 years before, I suggested his expectations had been fulfilled; radiata pine is arguably the most domesticated conifer, well-characterised genetically; it remains the single most important forest plantation species in the southern hemisphere and one of the most important globally; and advances in control-pollination and vegetative propagation allow the rapid capture of gains and the application of biotechnologies. For these reasons, radiata pine has the characteristics of a model species, and could be said - rephrasing Bill's words - to be assuming the status of a paradigm of tree improvement. To the extent this is so, it adds significance to the issues, challenges and progress reported in these proceedings.

Amongst the major points of interest, concern or discussion were:

- our emerging understanding of the likely evolutionary history of radiata pine, which suggests a more dynamic metapopulation structure than hitherto appreciated;
- the spread of pitch canker in California, which is limiting the value of *in situ* resources for *ex situ* purposes, though (looking on the bright side!) offering opportunities for resistance screening of exotic populations within the native range;
- the diminishing viability of seeds remaining in store from the 1978 rangewide collection which necessitates planting soon, or foregoing those genetic resources;
- how controlled pollination and vegetative propagation systems have advanced to the stage where they impose few constraints on breeding and deployment strategies; whilst storage and rejuvenation technologies - though advancing - are not yet at that stage, radiata breeders and growers already have a wide portfolio of both propagation and deployment options;
- how best to understand and address the real and perceived risks associated with the expansion of clonal and, ultimately, transgenic forestry;
- a renewed focus on the derivation of breeding strategies and objectives to deliver end-product value, thus emphasizing the role of wood quality traits;
- a concomitant shift towards reconstituted and engineered wood products, challenging conventional concepts of wood quality;

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- notwithstanding substantial progress in both arenas, some perception on the part of the molecular geneticists that their quantitative colleagues had delivered “more strategies than gains”, and some scepticism in the reverse direction about the “speculation: fact ratio” associated with molecular biology;
- nevertheless, evidence of increasing synergies between quantitative and molecular genetics, with growing appreciation of the insights each offers on the other, of the complementarities and tradeoffs between them, and of the associated strategic implications and dilemmas.

The principal themes I saw running through the papers and discussions were:

- a much stronger strategic focus on end-product value, and on cost-efficient delivery of gain. Technological and market change complicate the former, as the different costs, benefits and risks do the latter - although we have better information and tools to explore the options in both cases;
- continuing effort and real progress in the development and application of biotechnologies, complicated variously by technical, strategic and proprietary issues;
- a sense of the potential for genetic resource loss, for disease outbreaks, and for effective opposition to the deployment of transgenic trees - each of which suggests at least the continuation, and probably the enhancement, of coordinated action to address these concerns;
- the magnitude of institutional challenges to those elements of cooperative and public-good research which, as Mike Carson’s welcoming statement reminded us, have been the basis of success in the past; we saw in this meeting, as elsewhere, examples of both synergies and tensions between the old and new models of research.

Bill Libby thanked the 1982 office holders and conference organisers for “an unusually effective and productive meeting”. The same sentiment is appropriate here: thanks to the Working Group’s Co-Chairs, Peter Ades and Mike Carson, and to Mike and his Organising Committee and their colleagues, for their efforts. Despite the impressive and intended longevity of many participants, we had better not wait another fifteen years for the third meeting of radiata geneticists; I understand our Chilean colleagues are already working to ensure we meet sooner.

Announcements

PCR Primers Derived from Mapped cDNA Clones of Loblolly Pine

Berhanu Temesgen(bt@s27w007.pswfs.gov)

The first set of PCR primers we developed from cDNA sequences and used to amplify samples of genomic DNA from two loblolly pine mapping populations (Devey et al., 1994, Theor Appl Genet 88: 273; Groover et al., 1994, Genetics 138: 1293) was posted at Dendrome (<http://s27w007.pswfs.gov/Data/berhanu.html>).

The file also contains results of screening for polymorphism of the PCR products using various restriction enzymes. The full version of this work is in press (Harry, Temesgen, and Neale “Codominant PCR-based Markers for *Pinus taeda* Developed from Mapped cDNA Clones,” Theor Appl Genet).

IMPACT: An International Venture to Study Pitch Canker in *Pinus radiata*

CSIRO Forestry and Forest Products, Australia has proposed a broad-scale initiative to establish *Pinus radiata* (Monterey pine or radiata pine) field trials in Northern California. The trials, referred to as the IMPACT trials, will serve as a resource for the study of pitch canker over the next 10 to 15 years. The principal research providers on the project will be CSIRO, Australia; Forest Research Ltd., New Zealand; Bioforest S.A., Chile; and the USDA Forest Service, Institute of Forest Genetics. Forest industry collaborators from Australia, New Zealand, and Chile will provide financial and in-kind support for the project.

A workshop of research and industry collaborators involved in the IMPACT project will be held in Monterey, CA on November 29 to December 3, 1998. Outcomes from the workshop will include an understanding of current research on pitch canker in radiata pine and a focused research plan for the IMPACT trials and follow on projects.

Further details on the project and workshop can be found on the web at: <http://www.ffp.csiro.au/tigr/radiata/impact/>.

Meeting Report:

Workshop on QTL Detection and Marker-Assisted Selection in Forest Trees
5th December 1997, Rotorua, New Zealand

Phillip Wilcox

Approximately 70 participants attended this workshop which was held immediately following the IUFRO conference on Genetics of Radiata Pine. Eight speakers presented results from a range of experiments and/or simulations. Phillip Wilcox (FRI, NZ) briefly introduced the topic, and pointed out some of the features relevant to marker assisted selection in forest trees, as well as some challenges and constraints. Steve Openshaw from Pioneer HiBred International gave the first of five presentations on QTL detection. Steve described results from early MAS experiments in corn, involving selection based upon marker trait associations detected with small population sizes. These results showed little or no gain from MAS. Subsequent results from a large QTL detection experiment indicated QTL effects in small populations were significantly overestimated, which could explain lack of positive results from early QTL detection experiments. Dave Neale (IFG, USDA, Davis, California) described results from a range of mapping experiments in loblolly pine and Douglas fir. In loblolly pine, marker-trait associations for components of radial wood density profiles had been found, and verification populations have been established to confirm these associations. Similarly, in Douglas fir, associations have been found for bud phenology, and establishment of a verification population is in progress. Karen Aitken (ForBio Research, Australia) presented results from QTL detection experiment with a full sib family of radiata pine using RAPD markers segregating in a pseudotestcross manner. Associations between markers and a range of traits were detected, including several interactions between

genomic regions. Sue Carson (FRI, NZ) described results from a selective genotyping experiment in radiata pine where a marker-trait association was detected in the tails of a phenotypic distribution, and subsequently verified in an independent population, although the magnitude of effect was much less in the verification population. Satish Kumar (FRI, NZ) described results from his Ph.D. research where he has developed theory for QTL detection in large outbred pedigrees with multi-allelic codominant markers. Satish showed that such markers are significantly more powerful than biallelic markers, irrespective of trait heritability.

The afternoon session focused on results from marker assisted selection experiments or simulations. Jeanne Romero-Severson (Perkin Elmer AgGen Ltd) presented results from a MAS experiment for tomato paste production, where significant gains had been achieved in a relatively short period. Sue Gardiner (HortResearch, NZ) described features of MAS in apple, including some successful double blind experiments involving prediction of disease resistance based upon markers linked to a single dominant resistance gene. Sue pointed out that while apple geneticists have had considerable success with major resistance genes, less progress has been made with quantitative traits, and apple geneticists could learn much from forest geneticists who have spent considerable effort on mapping complex quantitative traits. The final talk was given by Peter Carter (Carter Holt Harvey Forests Ltd) who described a cost - benefit analysis of marker assisted selection in radiata pine.

Even assuming small - moderate sized QTL effects and relatively large QTL detection and verification populations, results indicated that MAS within families of radiata pine could yield significant commercial gain. Final comment and wrap-up (and prizes for speakers) was given by Barbara McCutchan (Westvaco, USA).

Several talks relating to QTL detection and MAS were presented during the conference on IUFRO Genetics of Radiata Pine which was held before the workshop. Richard Kerr (Southern Tree Breeders Association, Australia) described some preliminary results from simulation studies indicating similar levels of gain could be obtained from clonal forestry and MAS. Phillip Wilcox (FRI, NZ) reviewed results in radiata pine from a range of QTL detection experiments. Sample sizes in these experiments tended to be small, and only some experiments included an independent verification step which used populations derived from the same pedigree. Results generally showed the magnitude of marker-trait associations in verification populations were usually much less than in the initial detection population, although the direction of association tended to be consistent. Glen Dale (ForBio, Australia) outlined a conceptual framework for altering breeding strategies by incorporation of marker-trait information and accelerated flowering into existing breeding programs.

Several themes were evident from the both the workshop and related talks during the IUFRO conference:

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Research on the Molecular Genetics and Biochemistry of Phenylpropanoid Metabolism in Trees at the University of British Columbia, Vancouver, Canada



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Our laboratories at the University of British Columbia (UBC) have been collaborating for the last several years on fundamental and applied research projects aimed at understanding the control of phenylpropanoid metabolism in woody species. John Carlson, our colleague and collaborator on some of these projects at UBC, has recently accepted a new position at Pennsylvania State University.

The phenylpropanoid pathway provides plants with a mechanism for the diversion of large amounts of carbon from aromatic amino acid metabolism into the biosynthesis of products based on a phenylpropane skeleton. The products of phenylpropanoid metabolism provide highly efficient UV screening (flavonoids and small phenolic compounds), control of water loss and structural rigidity (lignin), and defense against pathogens (many phenylpropanoid compounds), all traits that are essential for the success of land plants. Thus, the evolution of phenylpropanoid pathways likely played a key role in the evolution of plants capable of colonizing the land environment. The major phenylpropanoid natural product in trees is lignin, a major component of secondary xylem. Other phenylpropanoid compounds in trees probably play important defensive roles against pathogens and pests, but these have been little studied in trees relative to herbaceous plants. We have been investigating specific aspects of phenylpropanoid metabolism in poplar and selected gymnosperm trees with the goals of 1) understanding the biochemistry and regulation of phenylpropanoid metabolism in these trees and 2) manipulating lignin subunit composition and/or quantity.

Phenylalanine ammonia-lyase (PAL)

All phenylpropanoid biosynthetic pathways, including that leading to lignin biosynthesis, can be considered to originate with the activity of the enzyme phenylalanine ammonia-lyase. This reaction diverts carbon from the protein amino acid phenylalanine into the multi-step phenylpropanoid pathway that eventually leads to formation of the lignin

polymer, flavonoids, and a large number of other phenolic compounds. In all angiosperm species examined to date, *PAL* is encoded by a multi-gene family and it was therefore surprising when *Pinus taeda* was reported to contain a single *PAL* gene. One of the possible implications of such a unique pattern could be differences between gymnosperms and angiosperms in the mechanism used to regulate the lignin biosynthetic pathway. To verify this reported distinction, we took advantage of the presence of several strongly conserved amino acid sequences within known *PAL* genes to develop a PCR-based screen of *Pinus banksiana* genomic DNA isolated from a single megagametophyte. This study revealed that the *P. banksiana* genome contained at least five different *PAL* genes, and demonstrated that all of these loci were transcribed to differing degrees in various tissues and/or developmental stages. There is therefore no *a priori* need to invoke any different models for phenylpropanoid pathway regulation in the two major taxa of woody plants.

On a phylogenetic level, our studies of *PAL* in conifers and other organisms have revealed that this enzyme represents a very ancient biocatalyst, whose origins are probably closely linked to the original evolution of a capability to colonize the primordial landscape, as discussed above. The only protein showing a phylogenetic relationship with *PAL* is histidine ammonia-lyase (*HAL*), whose occurrence in plants is uncertain, although it is found in groups as diverse as bacteria and mammals. We are presently exploring the evolutionary relationship between *PAL* and *HAL* at a functional and DNA sequence level to gain insight into origins and derivation of the modern proteins from the putative ammonia-lyase progenitor. In poplar and aspen, *PAL* is also encoded by a gene family that contains at least four members. We have studied two poplar *PAL* genes (*PAL1* and *PAL2*) from *Populus deltoides*, *P. trichocarpa*, and their hybrids. RFLPs corresponding to these *PAL* genes were identified and used to place both genes on the *Populus*

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linkage map developed by Toby Bradshaw, Reinhard Stettler, and colleagues. Both genes are highly expressed in a sub-epidermal cell layer in young leaves and stems, where high levels of soluble phenylpropanoid compounds accumulate, but are more weakly expressed in secondary xylem. The *PAL1* promoter contains putative cis-acting regulatory elements conserved in all *PAL* genes examined to date. We have examined the expression of *PAL1* promoter-*b*-glucuronidase (*GUS*) gene fusions in transgenic tobacco and poplar. In tobacco, *PAL-GUS* expression is limited to developing xylem, while in poplar expression is very strong in young leaves and stems, and lower in older leaves and stems undergoing secondary growth. The latter expression pattern is similar to that of the endogenous *PAL* gene, suggesting that the promoter contains the information required to specify developmentally regulated expression in poplar, but that the developmental signals that activate *PAL1* expression differ in tobacco and poplar. We are interested in using the *PAL-GUS* poplar trees to follow *PAL* expression over the growing season under field conditions.

4-coumarate:coenzyme A ligase (4CL)

A critical step in the biosynthesis of a large number of phenylpropanoid natural products is the activation of the hydroxycinnamic acids (products of *PAL* and a number of hydroxylases and *O*-methyltransferases). Most frequently, activation is achieved by the formation of coenzyme A esters of these acids, reactions catalyzed by hydroxycinnamic acid:CoA ligase (more commonly referred to as 4-coumarate:CoA ligase, *4CL*). For example, in lignin biosynthesis 4-coumaroyl:CoA, coniferoyl:CoA, and sinapoyl:CoA, derivatives of the corresponding acids, are substrates for reduction to aldehydes by the lignin-specific enzyme cinnamoyl-CoA reductase (*CCR*). Further reduction to cinnamyl alcohols (monolignols) is catalyzed by cinnamyl alcohol dehydrogenase (*CAD*).

One of our interests has been to investigate the potential role played by *4CL* in regulating carbon flow into the different monolignol precursors of the lignin polymer. Like *PAL*, *4CL* is encoded by a gene family in poplar. It has been hypothesized that different *4CL* gene family members encode *4CL* isoforms with distinct substrate utilization preferences. Such isoforms could preferentially direct the biosynthesis of specific CoA ester intermediates, which would then be directed into the biosynthesis of specific monolignols. To test this, we have characterized in detail the cDNAs and corresponding recombinant proteins derived from two poplar *4CL* gene family members with divergent DNA sequences. Recombinant proteins from the two genes produced in baculovirus-infected insect cells showed identical substrate utilization profiles, and these profiles were in turn nearly

identical to those of *4CL* enzymes in crude protein preparations from developing poplar xylem. These results indicate that while distinct *4CL* isoforms are encoded by the poplar *4CL* gene family, all isoforms may have similar substrate utilization profiles and are unlikely to play a role in regulating carbon flow into the biosynthesis of different monolignols, as originally hypothesized. To test this further, we are currently using a PCR-based approach similar to that used for pine *PAL* genes to identify and clone more highly divergent *4CL* gene family members from poplar.

Recent investigations by ourselves and others show that sense and antisense-suppression of *4CL* activity in transgenic tobacco and *Arabidopsis* can significantly alter the subunit composition of the lignin in these plants. These results could be explained by the existence of an uncharacterized reaction(s), in addition to the *4CL*-catalyzed reactions, leading to monolignol biosynthesis, and/or by the existence of distinct metabolic channels leading to the biosynthesis of different monolignols. The results point to previously unrecognized complexity in the control of monolignol biosynthesis, and suggest that similar suppression of *4CL* activity in poplar and other angiosperm trees could result in predictable changes in lignin subunit composition.

Coniferin b-glucosidase (CG)

The activation of xylogenesis in conifer stems is accompanied by a rapid accumulation in developing xylem of coniferin, the 4-*O*-glucoside of the lignin precursor coniferyl alcohol. The spatial and temporal correlation of these events has led to a general model of lignin biosynthesis that proposes glucosylation of newly-synthesized coniferyl alcohol, followed eventually by re-mobilization of the alcohol through the action of a specific coniferin b-glucosidase, possibly in the cell wall where lignin polymerization takes place. If the model is valid, this cycle would make an attractive target for genetically engineered reductions in lignin accumulation with a minimum of pleiotropic effects. To test that concept, we have purified a coniferin b-glucosidase from *Pinus contorta* (lodgepole pine) and used the resulting amino acid sequence information to retrieve the corresponding cDNA. This gene is being tested in sense and anti-sense orientations in various species of transgenic plants to examine the impact of suppression and/or over-expression of the CG function on lignin formation and other traits.

At the same time, we have been attempting to locate a homologous CG function in hybrid poplar xylem, but this has proved remarkably elusive. It is not clear at this point whether this difficulty reflects a fundamental difference between conifer lignification and angiosperm lignification.