

1998 IEG Workshop - “Wood and Wood Fibers: Properties and Genetic Improvement”

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The Institute of Paper Science and Technology hosted the 1998 IEG Workshop, co-sponsored by International Paper and the Institute of Paper Science and Technology. The presentations were drawn from many disciplines and covered aspects of wood chemistry, fiber physics, genetics, and molecular biology.

The 1998 IEG Workshop, “Wood and Wood Fibers: Properties and Genetic Improvement” was held at the Institute of Paper Science and Technology, Atlanta, Georgia from 19th –22nd July. As appreciation grows of the potential of genetics and molecular biology for wood quality improvement, interdisciplinary collaborations are being established. The 1998 IEG Workshop, co-organized by Dana Nelson (International Paper Company) and John Cairney (Institute of Paper Science and Technology), brought together, from six countries, 97 scientists whose different disciplines are being applied to understanding and altering wood characteristics. Sessions covered industrial perspectives, reviews of the structures of fibers and the lignin and how they affect product quality, plus recent advances in chemical and molecular analysis, and how breeding and silvicultural practices can optimize quality gains. We are grateful to the session moderators for providing drafts for the summaries given below. More information on the conference, including abstracts of the presentations, can be found on the IPST web page at <http://www.IPST.edu/treeconf/>.

General Session I: A Global View

Moderator: Nick Wheeler (WheeleN@wdni.com)

Weyerhaeuser Company

The session opened with a welcome and introductory talk by Dr. James Ferris, (Institute of Paper Science and Technology). Dr. Ferris’s presentation, entitled, “**The impact of wood property improvements and R&D needs on the pulp and paper industry**”, helped set the stage for the rest of the meeting by outlining the influence of various demographic factors on global wood and paper consumption and how technology can play a role in meeting projected future needs. Dr. Ferris points out that major battles are shaping up between current and new producers of pulp and paper, with the perceived efficiency advantages favoring recent entries. These advantages can be attributed to application of new technologies in forest management, genetic manipulation and milling, in areas with high growth potential. For current producers in the northern temperate zone to stay competitive, strategic research needs must include: development of faster growing, higher yielding softwoods; use of selected hardwood opportunities; and application of wood quality improvements to reduce costs and increase productivity. Dr. Ferris worked through

hypothetical examples of how changes in wood characteristics, such as lignin content, could provide advantage to companies in Northern latitudes who are currently in competition against enterprises utilizing rapidly renewable fiber supplies. Dr. Ferris concludes that major shifts in the industry will occur as a function of wood availability and quality.

Dr. Robert Kellison, (Champion International), gave the second talk entitled, “**Wood property research needs associated with fast growing plantations**”. Bob detailed observations from his extensive travels around the world, from the vast but under-managed and under-utilized forests of Siberia to the rapidly expanding, fast-growing plantations of the Southern Hemisphere. Dr. Kellison notes a number of factors common to the most successful new wood producing operations. These include:

- Intensive plantation management and short rotations;
- The ability to produce, evaluate, and utilize clones.
- Rapid plantation turnover, clonal replacement capability, and high wood utilization efficiencies.

Dr. Kellison suggests that wood quality traits are becoming increasingly important in short rotation plantations, with emphasis on traits such as fiber count and microfibril angle. Biotechnology will almost certainly play an important role in addressing certain issues, in particular traits such as disease resistance or environmental hardiness.

The talk, “**Pine genomics and increasing wood supply in the southeastern United States**”, was presented by Dr. Claire Williams, (Texas A&M University). Claire highlighted the growing forest genetics “stewardship” issue and the difficulties encountered in the integration of tree improvement and molecular genetic technologies. Along with other speakers at this conference, Claire pointed to the rapid advances being made in biotechnology and the concomitant increase in intellectual property protection and sale. Alliances being made today in pine genomics studies will have an economic impact on all of us for years to come.

Dr. Williams recounted the history of forestry in the South, from the exploitation of our forests for naval stores and supplies beginning in the 1600’s until plantation establishment began in the 1930’s. She pointed out that far-thinking scientists such as Hans van Buijtenen were promoting wood properties as important goals of tree improvement as early as the 1950’s and 60’s.

The talk briefly addressed the rate of evolution within the pine genome, and the rapid expansion of genomics level research. The question of how genes are dispersed within genomes remains to be determined and the presence of multi-gene families constitutes both problems and opportunities. She concluded with a brief description of the ongoing research activities in the lab at Texas A&M.

General Session II: Physical and Chemical Properties

Moderator: Barbara McCutchan (BGMcCut@Westvaco.com)

Westvaco Corporation

Paul Kibblewhite (PAPRO New Zealand), emphasized **microstructure characteristics for product specification**, using his extensive expertise with *Pinus radiata* as a basis for the wood fiber characteristics associated with kraft pulp and paper quality. Paul stated that wood density is not always a good predictor of handsheet bulk. He showed a cross-sectional diagram of two

woods (i.e. his Super Duper slide), each with the same basic density and wood volume. One wood resulted from many, thinner-walled fibers while the other had fewer, thicker-walled fibers. Density was not a good predictor of fiber quality or the suitability of a tree for a given paper grade. He stated that knowledge of fiber dimension and fiber length allows one to predict handsheet bulk and more importantly, product suitability.

Cell wall thickness and tracheid length measurements have been made using SilvaScan, a machine developed by Evans at CSIRO, Australia. They have found good correlations of breast height results with whole tree values and found high broad sense heritabilities for tracheid perimeter and wall area, 0.89 and 0.79, respectively, with eleven 16-year-old clones. The critical wood properties for kraft pulp were given as basic density, lignin to cellulose ratio, fiber length and fiber dimension. They advocate tailoring the wood for the end product, utilizing both wood processing segregation by wood age and site, since 1980's, and genetic selection for specific fiber type, starting soon.

Don Dimmel (Institute of Paper Science and Technology), **provided technical discussion on the differences between hardwood and conifer lignin structure** (and the effects of these differences in pulping reactions). He reviewed the fate of lignin, cellulose and hemicellulose during pulping and bleaching and the effect of different chemicals upon yield, energy use and pulp strength. He presented that hardwood lignin is easily fragmented under pulping conditions because of the presence of syringyl monolignols, which are lacking in softwood lignin. Syringyl lignin has fewer C-5 (carbon to carbon) linkages than guaiacyl lignin, making it more susceptible to chemical fragmentation during pulping. In addition, fragmented syringyl units undergo less recombination due to blocked C-5 units.

Dr. Dimmel discussed traits that, if altered, would have considerable impact on the Paper Industry. These included the alteration of lignin quantity and quality, increase in cellulose, a decrease in hemicellulose and the production of specific hemicelluloses. Certain hardwood trees contain naturally occurring pulping catalysts, Anthraquinones, whose alteration, by genetic means, in terms of level or nature, would be of considerable benefit.

General Session III: Measurement Technologies

Moderator: Bob Purnell (Robert.Purnell@IPaper.com)

International Paper Company

This session focused on technologies to more accurately and efficiently measure important wood and wood fiber characteristics. Three papers were presented dealing with measuring wood density, chemical composition, and microfibril angle. Dr. Gerald Tuskan (DOE, Oak Ridge National Laboratory) discussed the work he and others were doing on **determining wood density using computer-assisted x-ray tomography (CT)**. Using CT, wood samples are scanned from multiple directions so that a 3-D view of each sample can be reconstructed in a computer. Samples do not need to be milled and multiple samples can be scanned at the same time. Dr. Tuskan showed results from 4-year-old hybrid poplar clones and discussed the possibility to increase pulp yield per acre through selection of clones with desirable wood characteristics. Efforts are underway to link wood density phenotypes to molecular markers contained within the *Populus* genetic map.

Dr. Mark Davis (DOE, Renewable Energy Laboratory) discussed the work he and others were doing on **determining wood chemical composition using pyrolysis molecular beam mass spectrometry and multivariate analysis (Py-MBMS)**. This technique measures the lignin, cellulose, and hemicellulose contents in a fraction of the time required by traditional wet lab chemistry. Data from hybrid poplar and loblolly pine showed high correlation between Py-MBMS and wet lab analysis. Other methods that can do similar analyses include FT-IR, mass, NMR, and NIR spectrometry.

Dr. Robert Megraw (Weyerhaeuser Company) discussed the **measurement of microfibril angle using x-ray diffraction calibrated by light microscopy**. Microfibril angle changes throughout the S₂ cell wall layer. Within a fiber, the microfibril angle can change as much as 8 degrees, and among fibers within the same ring it can differ as much as 21 degrees. Many fibers must be measured to obtain an accurate and precise assessment of microfibril angle. Microfibril angle is also a function of both height and rings from the pith. A single wood sample from breast height may not be representative of the whole tree. Whereas light microscopy measure individual fibers and can be used for both wood and pulp, it is a slow and subjective method of analysis. X-ray diffraction can be used to quickly measure mean microfibril angle after a calibration curve is developed, the technique is more objective, all fibers are seen, though it is only suitable for solid wood.

Concurrent Session I: Poster Session

Coordinator: Barb Crane (Barbara.Crane@IPaper.com)

International Paper Company

The Monday afternoon first concurrent session consisted of five poster presentations emphasizing different aspects of genetic improvement of wood properties.

Two posters from our international friends (H. Almeida et al. and C. Pionneau et al.) were presented by Guillaume Chantre (AFOCEL, Nangis, France), who traveled from France to join us. The first poster **described a new project being launched that will consider market end-user requirements in determining selection and breeding of desired wood properties**. These insights will help tree breeders and wood users improve efficiency in selecting the appropriate genotype for the desired end product. A combination of traditional and modern methods (such as DNA markers) will be used to identify the properties in each of the three species being studied. The second poster **discussed the process of studying genes and proteins involved in the biological process of wood formation**. The researchers used 2D-PAGE to identify differences in xylem protein expression of normal and compression wood. This information could lead to associations with candidate genes responsible for differing wood properties and ultimately be useful in marker-assisted selection of desired wood traits.

The remaining contingent of posters was presented by various members of IPST. H. Meng et al. **presented an interesting scenario on the potential benefits of genetically engineering forest trees for increased endogenous Anthraquinone production**. Higher levels of this compound would increase pulping efficiency. In addition, if the plant could synthesize it naturally, it would decrease manufacturing costs and lower adverse environmental impacts. This group has successfully isolated the isochorismate synthase gene, an important regulatory gene in the Anthraquinone synthesis pathway. It has been recombinantly expressed in *E. coli* and further testing is planned.

S. Van Winkle et al. illustrated the competitive absorption of varying lots of activated charcoal in tissue culture growth media. It appears that this is an influential factor in the optimum absorption of critical hormones. Sucrose concentration and glutamine also play a role in this process. The study materials were Norway Spruce embryos.

The final poster, presented by P. Montello et al. won the **BEST POSTER AWARD** at the workshop. The theme addressed the importance of proper cryopreservation of loblolly pine somatic embryogenic cultures, the hazards and pitfalls. This process involves long term storage of material in liquid nitrogen (-196°C), recovery from storage and testing for viability and survivability. These researchers have modified current protocols, achieving better survival of cultures and lower costs.

Concurrent Session II: Short Oral Presentations

Moderator: Tom Byram (Byram@tam2000.TAMU.edu)

Texas Forest Service

The Monday afternoon second concurrent session consisted of four short volunteer talks emphasizing four different aspects of wood quality. The range of topics illustrated the contributions that fields as diverse as biochemistry, wood science and clonal forestry must each make in order to adequately address wood quality in real world situations.

C. P. Joshi (Michigan Technological University) discussed the discovery and characterization of two S-adenosyl-methionine dependent O-methyltransferases (OMTs) from loblolly pine. The methylation of hydroxycinnamic acid derivatives in lignin biosynthesis was previously believed to be carried out by a single OMT that led to production of guaiacyl rich lignin typical of gymnosperms. Dr. Joshi and his colleagues have now shown that **loblolly pine has a novel multifunctional OMT, AEOMT, that catalyzes the methylation of hydroxycinnamic acids as well as hydroxycinnamyl CoA esters.** Moreover, another structurally unrelated OMT, caffeoyl-coenzyme A O-methyltransferase, also plays an active role in wood formation. These enzymes offer attractive targets for regulation or modification in order to affect the lignin composition of pines.

Martin Maunders (Advanced Technologies (Cambridge) Limited) discussed field trials of genetically transformed *Eucalyptus*. Several *Eucalyptus* species were transformed with a constitutively expressed GUS gene. **Individuals of *E. camaldulensis* and *E. globulus* were subsequently established in field trials where it was demonstrated that genetic transformation caused no apparent deleterious effects in their development.** This leads to the expectation that it will be possible to incorporate novel genes such as those controlling wood quality traits into eucalypts without unwanted effects on adaptability or growth rate.

Milosh Ivkovich (University of British Columbia) reported the results from extensive morphometric analysis of spruce. He found that ring width and cells per ring had low heritabilities, while double wall thickness and cell size were moderately heritable. **Similar cell wall thickness to cell size ratios could be achieved by either modifying lumen size or by altering their cell wall thickness.** In addition to elucidating genetic relationships among several wood cell parameters, his paper emphasized the need for individual cell measurements where characterizing cell to cell variation is as important as determining overall averages.

Gary Peter (Institute of Paper Science and Technology) reported **on a relatively inexpensive and rapid method for measuring microfibril angle in the S2 layer of southern pines**. This method uses a high-resolution optical microscopy technique, Differential Interference Contrast (DIC), to image microfibrils. The DIC technique works well on the relatively thick cells of loblolly pine and gives results comparable to polarizing microscopy. Developing accessible, inexpensive technology that can be applied to large numbers of samples will be essential to understand the underlying components of wood quality.

General Session IV: Molecular Biology and Genetic Engineering

Moderator: Jeff Dean (JeffDean@arches.UGA.edu)

University of Georgia

The first presentation of this session, provided by Dr. Vincent Chiang (Michigan Tech University), was an immediate wake-up call to the morning audience. Using an antisense version of a xylem-specific gene encoding 4-coumarate:CoA ligase (4CL, the enzyme that diverts phenylpropanoid units into lignin biosynthesis and away from flavanoid production), Dr. Chiang's group has produced transgenic aspen trees that grow faster, contain more cellulose and have a greatly reduced lignin content. These results suggest that the biosynthetic pathways for cellulose and lignin are more interwoven than is commonly appreciated. **Micropulping experiments suggest that the transgenic aspen trees could yield up to 7% more cellulose while at the same time requiring a substantially reduced input of pulping chemicals**. The trees also showed enhanced root growth suggesting that propagation by rooted cuttings might also be improved. Of course, field testing to determine whether this genetic manipulation has affected other growth parameters, such as drought tolerance, and resistance to pests and windthrow damage, is a matter for future study.

Further surprises arising from major perturbations of the lignin biosynthetic pathway were next discussed by Dr. John MacKay (Institute of Paper Science and Technology), who described some of the characteristics of a loblolly pine tree that is homozygous for a null allele of the gene encoding cinnamyl alcohol dehydrogenase (CAD, the enzyme which catalyzes the final reductive step in the production of lignin precursors). Although the total amount of "lignin" in this mutant remained relatively unchanged from wild type, the subunit composition was changed so dramatically that it might be hard to classify it as lignin under some classical definitions of the polymer. With assistance from specialists in multi-dimensional NMR (J. Ralph) and pyrolysis-mass spectrometry (J. Boon), lignin from the mutant was shown to be greatly enriched in cinnamaldehyde and dihydroconiferyl alcohol, the latter a component of pine lignin that had not been previously detected. Using 1.0N NaOH as solvent, there was a 10-fold increase in the extractability of phenolic materials from the wood of the mutant tree with a concomitant 33% reduction in residual lignin. **This translated to more efficient pulping of loblolly pine wood using a soda process, but surprisingly not when a kraft process was used.**

Taken together, the first two talks underscored how poorly we understand what has sometimes been described as "the best understood biosynthetic pathway in plants," while at the same time hinting at just how wide the range of possibilities might be for manipulating lignin to commercial ends.

An ever-expanding range of exotic genes has the potential to be used in genetic engineering efforts to modify wood quality if we only had the means to limit their expression to differentiating wood fibers at the proper stage of development. Dr. Carol Loopstra (Texas A&M University) described her lab's efforts **to define the regulatory elements that confer tissue-specific expression to several classes of genes whose products are important for wood formation in both pine and poplar**. Her studies of two arabinogalactan proteins (AGPs), which are preferentially expressed in differentiating loblolly pine xylem, have shown that important regulatory sequences exist both upstream and downstream of the AGP coding sequences. This is in direct contrast to most other genes described to date for which the regulatory elements most often exist exclusively in the promoter region upstream of the gene. Making Dr. Loopstra's studies of pine promoters particularly difficult was the lack of an accessible transformation system for pine; the AGP regulatory elements did not function in the same manner when used to drive expression of transgenes in other plant species. Thus, the audience was reminded of the need to develop a greater array of molecular tools if we are to use genetic engineering to manipulate pine genomes in meaningful ways.

After a short caffeine interlude, Dr. John Cairney (Institute of Paper Science and Technology) presented the first of two talks that demonstrated how the rapidly evolving field of functional genomics will drastically change the manner in which we approach problems in tree biology. Dr. Cairney and co-workers have **used a PCR-based technique, called differential display, to identify several hundred genes whose expression varies during the development of zygotic and somatic loblolly pine embryos**. Placed in a high-density array on a solid membrane support, the cDNAs representing these differentially expressed genes were used to rapidly assess whether or not particular changes made to tissue culture media resulted in embryos whose gene expression patterns more closely matched the patterns seen in zygotic embryos at a similar morphological stage of development. These genes then become indirect markers for selection of new and improved media formulations. The changes in gene expression pattern can be scored within a few days, in contrast to the months it often takes before a new culture medium may be scored on the basis of embryo morphology, thereby greatly accelerating the pace tissue culture development. In addition, because the sequences of many of these genes have been conserved during evolution, the gene arrays can be used to study embryo development in other conifers, and possibly in more divergent species.

The final presentation for the session was made by Dr. Ross Whetten (North Carolina State University), who **described the development of an Expressed Sequence Tag (EST) database from cDNAs selected at random from a library representing the genes expressed in the developing wood of loblolly pine**. The online database, which currently includes nearly 2000 ESTs, is maintained at the University of Minnesota (E. Retzel) and may be accessed on the Web using the following URL (<http://www.cbc.UMN.edu/ResearchProjects/Pine/index.html>). With funding from the Agenda 2020 program, a further 10,000 EST should be added to the collection over the next three years. Analyses of the current collection have found that the genes may be roughly categorized as those related to genes of known function (25-30%), those related to other genes in the database but having unknown function (25-30%), and those showing no similarity to previously described genes (40-50%). These numbers are in line with those found in genome programs investigating other species. Using the more refined approach of constructing

subtractive hybridization cDNA libraries, these researchers are working to identify cDNAs representing genes that are differentially expressed in juvenile vs. mature and compression vs. side wood. Such genes could potentially be used to moderate the decreases in wood quality associated with these phenomena; however, as noted by Dr. Whetten, the limiting step in applying these tools may be in identifying the true physiological role played by the differentially expressed genes.

General Session V: Genetic Variation and Tree Improvement

Moderator: Steve McKeand(McKeand@cfr.cfr.NCSU.edu)

North Carolina State University

The final session of the 1998 IEG-40 Workshop emphasized operational tree improvement and research to facilitate the inclusion of wood quality traits into breeding programs. Bill Lowe (Western Gulf Forest Tree Improvement Cooperative) summarized opportunities that are available for including Wood Quality traits into breeding programs for specific products. **Quite often a desirable wood quality trait for one product may not be valuable or can even be detrimental for another product.** Another complication is that technology in the forest products industry changes so quickly that breeders must use a "crystal ball" to predict what mills will need in 25+ years.

Fortunately, the genetic variation for most Wood Quality traits are moderately to highly heritable so breeders can readily incorporate them into tree improvement programs. Complications have arisen from variation in natural stands not being the same as variation in common garden experiments, especially when species are used as exotics. In addition, unfavorable genetic correlations between growth traits and wood density often exist. One benefit from breeding for traits such as straightness, branching, and disease resistance, has been the improvement in quality of both solid wood and pulp and paper products.

An interesting example was given with wood density versus volume production. When the breeding goal is to increase dry weight production, volume is by far the more important trait. However, when selection indices are used to select trees that would reduce pulping costs, the weight for wood density compared to stem volume dramatically increase to 8:1. Tree breeders must carefully consider the breeding and product goals when considering wood quality traits.

Paul Belonger (The Timber Company) presented results from a large provenance/progeny trial with loblolly pine in the southeastern United States. **Provenance variation for volume traits was very large and was generally moderate to small for wood property traits** that include whole core densities (both gravimetric and x-ray densitometry) percent latewood, tracheid length and diameter, lumen diameter, and cell wall thickness. **Family differences were generally moderate to high within provenances for all traits.**

Unfavorable genetic correlations between volume and wood density were evident at the provenance level, but not within provenance. There was a larger unfavorable environmental correlation between volume and wood density across sites. Slower growing flatwoods sites had much higher wood density, probably because of availability of water later in the growing season resulting in higher percentage of latewood. The importance of site effects and the likely influence of silvicultural treatments such as fertilization, thinning, and pruning, were emphasized by both Paul Belonger and Bill Lowe.

These data will be valuable as breeders in the southern US begin to incorporate wood density, fiber characteristics, and wood uniformity measures into deployment and breeding programs for loblolly pine.

John King (British Columbia Ministry of Forests) summarized the new research emphasis on wood quality (WQ) traits for western hemlock in British Columbia. **With more emphasis on product quality and with improved measurement techniques, evaluation of WQ traits for solid wood and pulp and paper products has begun.** Virtually all the WQ traits had high heritabilities and sample sizes needed to detect family differences were small (< 8 trees/family). Unfortunately, there are some serious unfavorable genetic correlations between diameter and wood density ($r_G = -.5$) and between diameter and fiber coarseness ($r_G = .4$). If these relationships are ignored, serious product degrade will ultimately result.

More work is underway to evaluate the impact of selecting for specific fiber trait on paper quality and lumber quality. In micropulping trials, there appeared to be very good association between fiber traits and various handsheet measures, suggesting that more expensive testing of handsheets may not be needed.

Mitch Sewell (USDA Forest Service) updated the group on the Forest Service's wood quality quantitative trait loci (WQ-QTL) mapping project at the Institute of Forest Genetics in Placerville, CA. New analytical methods for evaluating 3-generation outbred pedigrees have improved QTL detection, and more traits are being assessed. Wood density and wood chemistry traits (percentage lignin, cellulose, hemicellulose, and extractives) are being genetically mapped. **Some "hot spots" in various genetic linkage groups where more than one trait has been mapped are suggestive that the true molecular basis of control of wood quality variation can be found.**

Several verification experiments have begun to determine if the same QTL will be found in other progeny from the same pedigree and from other related and unrelated pedigrees. These trials will be extremely valuable as breeders begin to incorporate marker/QTL associations (marker-assisted selection) into tree improvement programs.

Finally, Hans van Buijtenen (Texas A&M University) gave a wonderful talk about wood quality variation in the genus *Hypoplatanus*. Hans described opportunities for improvement in wood products both from genetic and silvicultural manipulation. Although, the tone was light-hearted and the species were fictitious, the message was very timely and clear. **With so much genetic variation and the powerful tools available (traditional selection to marker-assisted selection and genetic engineering), breeders have an incredible opportunity to improve wood properties and dramatically impact product value.**

Concluding Remarks: Paul Kibblewhite

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Dr. Kibblewhite encouraged the workshop attendees to move forward on the topics raised in the Tuesday afternoon small and large group discussion sessions. In two years' time we don't want to be discussing the same issues in the same way. Many of the current issues have been discussed for some time now in much the same way in many different forums around the world.

Most significant, Paul emphasized the importance and the continued need for good communication between the disciplines assembled here. People from all these fields-- molecular biology, gene mapping, silviculture, genetic engineering, tree breeding, quantitative genetics, wood chemistry and physics, instrumentation technology-- need to listen to each other and then act on this enhanced knowledge base.