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MODIFYING WOODY PLANTS FOR EFFICIENT CONVERSION TO LIQUID AND GASEOUS FUELS

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EXECUTIVE SUMMARY

The Short Rotation Woody Crop Program, U.S. Department of Energy, is using conventional tree breeding and intensive cultural practices to increase growth and yield of "energy plantations: of selected hardwood tree species. Large increases in productivity are being achieved. In view of these accomplishments, the agency is now evaluating opportunities for altering physical and chemical properties of biomass. Such research would seek to improve efficiencies of processes for converting lignocellulosic materials to ethanol and gaseous or other liquid fuels.

As a first step in preparing this report, importance of various traits to conversion efficiency was assessed. Literature and consultations indicated that cellulose and lignin quantity and quality are of major importance. Specific gravity and moisture content are somewhat less critical, whereas bark quantity and extractives are of minor consequence. Ash content and calorific value are least important. Maintaining utility for other uses and products is also critical. Suitability for uses other than energy production should ensure multiple markets thereby making investments in "energy plantations" more attractive to prospective growers.

After establishing relative importance of the several traits, literature pertaining to their genetic alteration was surveyed. Species now in use were emphasized. Review and subsequent synthesis evaluated prospects for meaningful improvement via both conventional selection and breeding and molecular or so-called biotechnological methods.

Findings indicate existence of genetic variation in all traits, but the range of variation generally is small. Both broad and narrow sense heritabilities are small to moderate, highly variable, and often derived from relatively narrow genetic bases. This is true not only for traits of complex nature or minor importance but also for major ones such as cellulose content. For many traits, available data are inconsistent and firm estimates of genetic control are lacking. Prospects for manipulating lignin content and specific gravity are more optimistic than those for altering other traits. Indeed, significant gains in such traits and cellulose content have been obtained or predicted, and these accomplishments should not be overlooked. Results also suggest that most traits of interest can be manipulated independently of others, including growth and productivity. More reliable estimates of genetic variation, heritabilities, genetic correlations, and genotype X environment interactions nevertheless are needed. More and better data would facilitate both conventional and molecular approaches, but improvement of traits specified above can be obtained now via conventional selection and breeding.

Information on biosynthetic pathways and potential intervention via molecular genetics indicates that lignin metabolism is most vulnerable to qualitative/quantitative manipulation. Knowledge or regulation/control of polysaccharide biosynthesis/deposition is insufficient to permit application of molecule approaches. Altering other constituents, e.g., extractives, hardly seems attractive, given our presently weak understanding of biosyntheses, inheritance patterns, and potential repercussions. Opportunities for

altering lignin quantity or quality include phenylalanine supply, storage/transport forms of lignin precursors, the oxidant for polymerizing lignin precursors, and lignin-carbohydrate bonds. Enzymes involved in formation of low molecular weight lignin precursors have been studied in some detail, and offer perhaps the best option at present. Manipulating precursor supplies may be helpful, but altering control of polymerization and deposition could have greater impact on ease of conversion to fuels. Future research seems best concentrated on biosynthesis and deposition of lignin, cellulose, and hemicellulose. Biochemical factors underlying composite traits such as specific gravity, moisture content, extractives, and bark quantity/quality also require attention.

Molecular and so-called biotechnological approaches can be used to further goals of the Short Rotation Woody Crop Program now and in the future. The potential exists for manipulating traits of interest via insertion of foreign genes into species used or proposed for use in "energy plantations". Cloning genes, transferring them into target cells, regenerating transgenic plants, and confirming expression and transmission of the genes can be accomplished largely with existing technologies. Improved systems for regenerating plants would be helpful. The major hindrance to improvement via molecular genetics, however, is our poor understanding of genetic and molecular bases of most traits. Before lignin and cellulose quantity/quality can be manipulated meaningfully, enzymes controlling important reactions must be better identified and characterized. In turn, genes responsible for synthesis and control of these enzymes must be isolated, cloned, and made available for transfer. Clearly, much research is required before manipulation can be effected at will. Several molecular techniques nevertheless can be applied immediately. Prime examples include antisense RNA and Restriction Fragment Length Polymorphisms. Antisense RNA techniques, in particular, could be used to elucidate functions of enzymes involved in lignin biosynthesis, thereby facilitating work on modification of this important and vulnerable trait.

INTRODUCTION

The Short Rotation Woody Crop Program (SRWCP), Department of Energy, is developing woody plant species as sources of renewable energy. Much progress has been made in identifying useful species, and testing site adaptability, stand densities, coppicing abilities, rotation lengths, and harvesting systems. Conventional plant breeding and intensive cultural practices have been used to increase above-ground biomass yields.

Given these and foreseeable accomplishments, program leaders are now shifting attention to prospects for altering biomass physical and chemical characteristics, and to ways for improving the efficiency with which biomass can be converted to gaseous and liquid fuels.

This report provides a review and synthesis of literature concerning the quantity and quality of such characteristics and constituents, and opportunities for manipulating them via conventional selection and breeding and/or molecular biology. Species now used by SRWCP are emphasized, with supporting information drawn from others as needed. Little information was found on silver maple (Acer saccharinum), but general comparisons (Isenberg 1981) suggest composition and behavior similar to those of the other species. Where possible, conclusions concerning means for and feasibility of manipulation are given, along with expected impacts on conversion efficiency. Information is also provided on relationships to other traits, genotype X environment interactions, and potential trade-offs or limitations. Biomass productivity per se is not addressed, except in terms of effects that may be caused by changes in constituent quality and/or quantity. Such effects are noted to the extent they are known or can be estimated. Likely impacts of changes, however effected, on suitability for other uses, e.g., pulp and paper manufacture, are noted.

The opening section describes conversion processes used for production of ethanol fuel (Biochemical Conversion) and gaseous or other liquid fuels (Thermochemical Conversion), and those woody plant constituents likely to influence conversion efficiency. Attention is focused on those processes now considered favorable, and likely to be investigated most intensively in coming years (Anon. 1988).

Findings of the literature review and synthesis are presented in three subsequent sections, with the first concerning modification via conventional selection and breeding. The second details biosynthetic pathways underlying important traits and discusses the potential for manipulating them. The third describes molecular approaches or altering such pathways and associated traits in the near and long term.

Conclusions and recommendations concerning traits and methods are summarized within individual sections. In addition, a general summary of major conclusions is presented as the closing portion of the report. The literature cited section lists all references used in preparing the report. Copies of most references used in preparing the report are provided under separate cover. Large books and symposium proceedings,

often widely available, were not copied. Where appropriate, however, specific portions of such sources that were used in report preparation were copied and are provided.

CONVERSION PROCESSES: DESCRIPTION AND STATUS

PRODUCTION OF ETHANOL FUEL

Both enzymatic and acid hydrolysis have been investigated as means for producing ethanol from woody biomass. Enzymatic hydrolysis will receive the greatest research effort in the coming 5-year period (Anon. 1988), and this report concentrates on modifications to improve its efficiency. This further seems appropriate in that feedstock characteristics for both process types are similar.

Enzymatic hydrolysis involves pre-treatment to separate major constituents and isolate lignin, followed by saccharification, fermentation, and distillation. Future process research will focus on selection and modification or organisms/enzymes to increase yields, reduce sensitivity to ethanol concentrations, and raise reaction rates via greater temperature tolerance. Research on organisms that ferment pentoses is also under way.

Preferred feedstock characteristics include more cellulose, less lignin, and increased specific gravity. Lignin containing less oxygen, and more responsive to pretreatment is also desirable. Moisture and ash content are of lesser consequence, but reducing the latter would alleviate pollution and disposal problems. Importance of extractives seems uncertain, but lower amounts seem desirable. Lower bark quantities may be beneficial, but bark contains a significant proportion of carbohydrate that can be converted to ethanol.

PRODUCTION OF GASEOUS AND OTHER LIQUID FUELS

Several thermochemical processes have been or are being investigated. Development of reactors for generating gaseous fuels has received much attention. A variety of reactors are available and all operate more or less the same. Emphasis on gasification, however, is giving way to accelerated research on conversion to liquid fuels via Catalytic High Pressure Pyrolysis and Low Pressure Fast Pyrolysis. As a result, this report emphasizes modifications to raise efficiency of liquification processes. Fortunately, similar characteristics are desired for all thermochemical processes. Without doubt, however, such requirements differ markedly from those for efficient ethanol production.

All thermochemical processes employ complex catalysts in early stages as well as in subsequent refining and reforming steps. Contents of constituents likely to foul catalysts should therefore be minimized. Desired characteristics include lower moisture content, higher specific gravity, more lignin, normal or lesser amounts of carbohydrates, and reduced oxygen content. Ash seems a neutral component, aside from potential

effects on catalysts and/or pollution. Extractive contents again represent an uncertain situation, but benefits could accrue from increasing amounts of those having high energy content and not likely to foul catalysts or cause disposal problems.

Reliable and large supplies of raw material on a year-round basis are important, and any modifications that reduce yields will be counterproductive.

FEEDSTOCK REQUIREMENTS FOR ENZYMATIC HYDROLYSIS

CHARACTERISTIC/CONSTITUENT	DESIRED MODIFICATION
Cellulose	Increase amount, decrease crystallinity.
Hemicellulose	Leave concentration as is or perhaps increase. Reduce number and length of side chains, raise solubility. Consider increasing hexoses at expense of pentoses. Minimize covalent linkages to lignin, and otherwise alter to facilitate enzymatic attack on cellulose.
Lignin	Reduce amount. Reduce guaiacyl to syringyl ratio. Consider other changes to simplify pre-treatment and precipitation as well as stimulate use.
Ash	Reduce amount. Eliminate heavy metals that could inhibit processing or cause pollution and disposal problems.
Moisture content	Leave as is, unless necessary to lower transport costs or alter cellulose crystallinity.
Extractives	Leave as is, or consider reducing concentration of those likely to inhibit processing or cause disposal problems.
Specific Gravity	Raise, but allow for negative correlations with other traits, especially growth. Also watch effect on lignin/cellulose ratio and access to cellulose.
Bark	Consider lowering bard to wood ratio or altering to a composition more like that of wood. May not be too important as carbohydrate content can be used.
Uniformity	Increase.

Note: Increasing efficiency of enzymatic hydrolysis could have negative impacts on thermochemical processes; e.g., less lignin would lower efficiency of pyrolysis.

FEEDSTOCK REQUIREMENTS FOR CONVERSION VIA PYROLYSIS

CHARACTERISTIC/CONSTITUENT	DESIRED MODIFICATION
Cellulose	Leave as is or diminish relative to lignin.
Hemicellulose	Leave as is or reduce relative to cellulose so as to lower overall oxygen content. Also, intermediates formed from cellulose are likely to be more useful.
Lignin	Raise amount. Reduce oxygen content. Change toward softwood form may be helpful.
Ash	Reduce amount. Minimize residues that could poison catalysts or cause pollution and disposal problems.
Moisture Content	Reduce to lessen energy needed for drying, improve process efficiency, and reduce transport costs.
Extractives	May want to increase, especially those with high energy content.
Specific Gravity	Raise, but allow for negative correlations with other traits, especially growth. Also watch effect on lignin/cellulose ratio.
Bark	Consider lowering bark to wood ratio or altering to a composition more like that of wood.
Uniformity	Increase.

Note: Prevalence of changes opposite those favoring enzymatic hydrolysis; e.g., call for more rather than less lignin.

PROSPECTS FOR MANIPULATION OF IMPORTANT CHARACTERISTICS AND CONSTITUENTS

CELLULOSE CONTENT

Carbohydrates comprising holocellulose are the starting material for ethanol production. Quantity and quality are therefore primary determinants of conversion efficiency. Alpha-cellulose, the primary constituent of holocellulose, affects wood fiber quantity and quality, and is critical for pulp and paper manufacture.

Hemicellulose, another major constituent, also affects energy and pulp production. Aspects likely to limit efficiency of conversion to ethanol include relative proportions of five- and six-carbon sugars, number and length of sidechains and covalent linkages to lignin.

Despite importance of this constituent, few workers have investigated genetic variation in and control over cellulose content, and information is not especially abundant.

Olson et al. (1984) evaluated holocellulose and alpha-cellulose contents of 75 clones of eastern cottonwood (Populus deltoides). Trees were grown at one location, and assayed 3 years after planting. Holocellulose content varied from 78.0 to 81.8 percent on an oven-dry extractive-free basis. Average content was 80 percent, and broad sense heritability was estimated at 0.08. Selection and breeding for this trait were not considered practical.

Variation in alpha-cellulose was somewhat larger (range = 48.2 to 55.8 percent), and averaged 51.1 percent, a value larger than those noted for many hybrid poplars. Broad sense heritability was 0.34, moderate and potentially large enough for practical improvement.

A strong and negative genetic correlation (-0.33) was noted between alpha-cellulose content and volume growth, indicating the difficulty of simultaneous improvement. Direct selection for growth, however, should cause only minor reductions in alpha-cellulose content. The authors favored combining the traits in future generations.

Genetic correlations between specific gravity and both holocellulose and alpha-cellulose were strong and positive (0.74 and 0.54, respectively), suggesting that selection for specific gravity will raise cellulose content. Phenotypic correlations (0.25 and 0.34) were lower, confirming the relatively low heritabilities and susceptibility to environmental influences. Since only one plantation was evaluated, information on genotype X environment interactions was not obtained.

Given the scarcity of information on genetic parameters, data from species not now used for energy production may clarify opportunities for, or limitations on, improving cellulose content. Zobel et al. (1966) found little variation in holocellulose content among 48 open-pollinated families of 5-year-old loblolly pine (Pinus taeda). The concluded that genetic control was absent or minor, but postulated that results could change with age or improved analytical methods.

In reexamining some of the same materials at 11 years, Jett et al. (1977) found that holocellulose content ranged from only 78.4 to 82.0 percent, and that family differences were not significant. Analysis, nevertheless, showed that some portion of total variation was heritable and associated with nonadditive effects. Lacking additive variation, the authors concluded that traditional mass selection and seed orchard approaches to improvement were not feasible, and suggested that the small nonadditive variation was not useful. Efficient technologies for exploiting it were unavailable at the time.

In discussing these and related results, Zobel et al. (1982) reaffirmed that little improvement could be obtained via approaches relying on additive variation, but argued that meaningful gains can be achieved via selection, breeding, and vegetative propagation.

Specifically, they cited the outstanding improvement obtained by Aracruz Florestal S.A. in Brazil with Eucalyptus spp. In addition to selecting for growth and pest resistance, this company selected for specific gravity and cellulose content. Substantial variability was found [Campinhos 1980, Zobel et al. (1982)]. Selections excelling in specific gravity were isolated to

raise amounts of wood substance per unit volume, and trees within this group having high cellulose content were chosen to increase cellulose yields per unit weight of dry wood substance. The authors considered the traits independent, and indicated that simultaneous selection was possible. Asexual propagation via rooted cuttings has yielded phenomenal gains in pulp yields per unit land area. Concomitant increases in uniformity have resulted in lower harvesting, processing, and manufacturing costs.

Blankenhorn et al. (1985a) evaluated the chemical composition of seven hybrid poplar clones from a single planting in central Pennsylvania. Variables, including holocellulose and alpha-cellulose, were quantified in wood, bark, and wood/bark samples collected from 1- to 8-year-old materials. Results indicated that wood typically had more cellulose than bark, and the wood/bark samples contained intermediate amounts. Wood, bark, and wood/bark holocellulose varied among ages within most clones, and also among clones at several ages. For some clones, holocellulose contents tended to increase with age, but trends were inconsistent. Similar results, though not statistically evaluated, were found for alpha-cellulose. Alpha-cellulose contents ranged from roughly 37 percent to a high of almost 49 percent across all ages and clones, with the perceived average at 3 years of age being substantially less than that noted for eastern cottonwood by Olson et al. (1985). The authors concluded that conversion efficiency would vary with clone, but more so with tissue type and age or time of harvest. In a companion study, Blankenhorn (1985b) evaluated chemical composition of three of the same clones growing on two contrasting sites. Wood, bark, and wood/bark samples were assessed at three different ages and from two consecutive coppice rotations. Holocellulose and alpha-cellulose contents of wood averaged 72.4 and 43.7 percent, respectively, across all clones, ages, and rotations. Effects of tissue type and age esser ially were the same as those noted above, and rotations significantly affected both variables. Holocellulose content of 4-year-old trees varied between sites, with differences narrowing across rotations. Alpha-cellulose contents generally differed from site to site, with the better site producing larger amounts. Nature and extent of differences among clones across sites apparently were consistent as no mention was made of genotype X environment interactions. In general, tissue age, rotation, and site influenced cellulose content more than genetic background. Regrettably, genetic structure of these experiments, i.e., clone number and nature, were not suited for quantifying genetic variation, inheritance patterns, or genotype X environment interactions, and results should not be construed as indicating that genetic control over cellulose content or other constituents is lacking. The greatest benefit of the structure was that extraneous variation was minimized, and useful comparisons of site, rotation, and tissue types were possible.

In an earlier test of the same seven clones, Bowersox et al. (1979) analyzed composite wood and bark samples from 4-year-old trees, and also samples of wood and bark that represented material of 1, 2, 3, and 4 years of age from one clone. Holocellulose content of oven-dry extractive-free samples varied significantly among clones. Contents averaged 67.1, and ranged from 58.4 to 74.7 percent, but the authors attributed much variation to factors other than genetics. In general, holocellulose content varied only slightly among locations within trees. In a related study, samples of the same clones were taken from a trial of different planting densities (Murphy et al. 1979). Neither spacing nor clone affected holocellulose or alpha-cellulose content.

Dickson et al. (1974) analyzed sugars in the third annual ring of 18 clones of several Populus species and hybrids. Clonal differences occurred for all sugars, except galactose. Glucose content, an indicator of alpha-cellulose content, ranged from 42.3 to 51.6 percent. Hybrids having P. nigra as one parent consistently had lower glucose contents, whereas contents of hybrids involving most other species occurred throughout the rankings. Such results confirm

earlier suggestions that Section Leuce poplars have low lignin and high cellulose contents, while those from Section Aegeiros have high lignin and low cellulose (Einspahr et al. 1968).

Earlier references on cellulose content give similar means and ranges (Zobel et al. 1966, Zobel et al. 1982, Jett et al. 1977, and Kenney et al. In press). Representative values for this and other traits, excerpted from Isenberg (1981) and Koch (1985) are summarized in the Appendix.

Dickson et al. (1974) found that xylose concentrations, an approximation of hemicellulose content, varied from 16.7 to 21.5 percent. Arabinose and mannose varied widely among clones, but the authors considered the variability inadequate for breeding. Bowersox et al. (1979) reported significant differences in pentosan content, another indicator of hemicellulose content, among seven hybrid poplar clones. The average was 16.2 percent, range = 12.5 to 18.7 percent. Such findings suggest that changing hemicellulose content may not be possible.

Modification of hemicellulose may not be necessary since better organisms and enzymes are being developed for conversion to ethanol. On the other hand, covalent linkages between hemicelluloses and lignin have been reported (Eriksson and Lindgren 1977, Joseleau and Gancet 1981). Such reports suggest attachment of lignin to arabinose and galactose in side chains of hemicellulose. While impact is unknown, such linkages may limit efficiency of both enzymatic and thermochemical conversion. Altering composition or prevalence of sidechains may increase ethanol yields, and yield more uniform products from pyrolysis. Effects on product yields and quality as well as ways to alter hemicellulose may warrant investigation.

In sum, genetic control of cellulose content seems nonadditive and weak. Improvement has been obtained in certain species, but long term breeding and asexual propagation are required. Several inconsistencies are apparent. Some reports show alpha-cellulose content varying directly with growth, while others suggest the opposite. Similar confusion exists for relationships to specific gravity. More data about such relationships and prospects for improvement are needed. Such data would benefit both producers of energy and manufacturers of pulp and paper. The many "energy plantations" established in recent years should provide a wealth of material for such investigations. If cellulose does indeed vary with growth, several avenues seem open to the conventional tree breeder. Increasing cell wall thickness and fiber numbers may raise cellulose content, and both variables can be altered via conventional selection and breeding. Fairly straightforward means are available for increasing rates of growth and duration of cambial activity. Prospects for manipulation via molecular genetics seem unlikely over the near term.

LIGNIN CONTENT

Lignin contains a higher proportion of carbon and hydrogen and less oxygen than cellulose. It therefore contributes much to the energy content of woody materials, and is well-suited to production of gaseous and liquid fuels. Quantity and quality, however, can reduce efficiency of ethanol production.

Similarly, more lignin means less cellulose and lower pulp yields as well as higher pulping and bleaching costs. Appropriate changes in quantity and/or quality could improve efficiencies in a number of processes.

Einspahr et al. (1968) summarized wood property data from several Populus species and hybrids. Differences among taxa in terms of lignin content were small (overall range = 15.7 to

22.9 percent). Younger trees tended to have slightly less lignin than older trees, but their lignin contents were quite uniform despite diverse parentage. The authors contended that potential for improvement was limited.

These same authors also evaluated lignin contents of 25 full-sib families of Populus tremuloides (Einspahr et al. 1967). Average content at 5 years of age was 18.1 percent; family means varied from 17.1 to 19.9 percent. Heritability, based on interclass correlations, was 0.58. The authors considered this estimate high and not especially reliable. Minor family variation suggested limited potential for genetic improvement.

Whitmore (1973) examined several physical and chemical characteristics of 11-year-old black locust (Robinia pseudoacacia) clones from an Ohio plantation. Lignin content averaged 22.30 percent, and ranged from 21.02 to 24.18 percent. Broad sense heritability was estimated at 0.17.

Lignin contents of 18 3-year-old clones from various Populus species and hybrids were analyzed by Dickson et al. 1974. Differences among clones were significant. Percent lignin averaged 21.3 over all materials, and ranged from 18.1 to 25.0. Lignin content and other chemical properties seemed independent of growth. Several clones having low lignin and high cellulose as well as rapid growth and disease resistance were identified. Given the variability in lignin content, the authors believed it under strong genetic control and worthy of consideration in breeding programs.

In a brief progress report, Anderson and Zsuffa (1982) cite examples of variability in hybrid poplar and willow Salix clones. Lignin content ranged from 19 to 27 percent in poplar, and from 26 to 35 percent in willow.

Blankenhorn et al. (1985a) analyzed lignin contents of wood, bark, and wood/bark samples from seven clones of hybrid poplar. Samples representing 1- to 8-year-old material were assessed. Bark contained more lignin than wood, and variation among ages was significant only for bark. Clonal differences were modest, especially for lignin content of wood. At age 8 years, e.g., percent lignin in wood ranged only from 17.05 to 21.85, whereas that in bark varied from 28.02 to 38.84. In a related test with three of the same clones, Blankenhorn et al. (1985b) found only minor variation among clones, rotations, and sites.

Kenney et al. (In press) give other data on lignin content, primarily from Populus and Salix species and clones. Means and ranges were similar to those noted above. Additional heritability values were not given.

In a novel twist, Wilcox and Smith (1973) devised selection indices to optimize genetic gain in various traits, move wood properties toward specific product objectives, and minimize variability in the desired properties. Data were obtained from 12-year-old grafts of 37 loblolly pines, and related progeny tests. Lignin content was estimated via specific light absorption coefficient, an indirect measure of lignin content (Wilcox 1973). Such indices deserve closer examination as both modified lignin content and greater uniformity would improve process efficiencies.

In reviewing opportunities for improving wood properties, Savidge (1985) describes variation of lignin content within trees, and provides examples of variation among species and provenances. Evidence, empirical and experimental, is also given showing that lignin content is not related to specific gravity and that genetic control of lignin and cell wall polysaccharides is

independent. He concludes that reducing lignin content without harming xylem development is feasible.

While opinions differ about feasibility of altering lignin content, prospects are tempting and significant benefits could accrue. Considering the millions of tons of wood pulped in the U.S. each year, even a one percentage point reduction in lignin content would have tremendous impact on pulp yields and costs. Taken together, the foregoing examples of genetic variability and associated heritability estimates plus the arguments of Savidge (1985) signal that exploratory research toward increasing, decreasing, or otherwise modifying lignin would be fruitful.

For conventional selection and breeding, the variety of material, especially interspecific hybrids, in breeding populations associated with existing "energy plantations" should prove easier in the long term. Opportunities for altering lignin quantity and/or quality definitely seem more appropriate and approachable than for other constituents, such as cellulose content. Indeed, several laboratories, e.g., Michigan Technological University and North Carolina State University, have initiated research on molecular approaches albeit in conifers. In addition, some workers are seeking to develop somaclonal variants with low lignin content (A. Hoffman, Oregon Graduate Center, personal communication).

SPECIFIC GRAVITY

Bulk is perhaps the greatest disadvantage of woody plant biomass as a feedstock for conversion to gaseous or liquid fuels or even as fuel for direct combustion. Packing more wood substance and/or energy into a given volume is therefore important to process efficiencies. This trait nevertheless may be correlated with other traits such as growth, and selection for specific gravity alone or in combination with other traits could lessen overall productivity, increase other constituents to undesirable levels, or lessen utility for other uses. Understanding inheritance of specific gravity, and its relationships to growth and other traits is therefore essential for meaningful manipulations.

Specific gravity is under moderate to strong genetic control in most tree species, and useful literature on narrow and broad heritabilities as well as correlations with other traits is available. Some assessments of genotype X environment interactions have also been made. To provide maximum but concise coverage of the considerable literature, means, ranges, heritabilities, and other genetic parameters from surveyed articles are listed in Table I. Only reports highlighting particularly important data, parameters, or relationships are discussed below.

Sennerby-Forsse (1985) evaluated specific gravity (g/cc) of wood from 1-year-old shoots of 20 clones from four Salix species. Samples were taken at 30 percent of stem height from shoots grown in each of three seasons. In the third season, average specific gravity was 0.34, with clone means ranging from 0.28 to 0.39. Variation among clones was significant, but considerable variability also occurred within clones. Those with the highest specific gravity ranked high in each of the three seasons.

Basic specific gravity of wood and bark from 2- and 10-year-old trees of hybrid poplar clones were examined by Anon. (1982). Within individual stems, wood density tended to increase from base to top, but the pattern was not especially consistent. Wood densities were comparable to those of older trees from native stands of various Populus species, but greater than that of 3-year-old poplar species and hybrids sampled by Dickson et al. (1974). For most clones, bark specific gravities exceeded those for wood.

TABLE I. Means, Ranges, & Heritabilities for Specific Gravity, Part A=Wood

Species	Age (Yr.)	Genetic Entries (#)	Means	Range	h^2 [1]	Authors & Year
Hybrid Poplar	3	18	0.334	0.272-0.368	-	Dickson et al. 1974
	2	10	0.397[2]	0.333-0.447	-	Sastry & Anderson 1980
	2	10	0.362	0.346-0.396	-	Anon. 1982
	10	5	0.362	0.328-0.408	-	"
	4	9	0.35	0.32-0.38	-	Phelps et al. 1982
	1	30	0.295	0.24-0.38	-	Anderson & Zsuffa 1983
	3	3	0.35[3](0.34)	-	-	Phelps et al. 1987
Eastern Cotton-wood	3	12	0.337g/cc	0.308-0.364	0.96BS	Herpka 1979
	3	117	0.335g/cc	0.285-0.389	0.86BS	"
	3	75	0.33	0.27-0.39	0.62BS	Olson et al. 1984
Aspen	5	25	0.37	0.343-0.402	0.42NS	Einspahr et al. 1967
	40-47	3	-	0.348-0.402g/cc	-	Yanchuck et al. 1983
	36	15	-	0.32-0.40g/cc	0.35BS	Yanchuk et al. 1984
Salix	1	20	0.34g/cc	0.28-0.39	-	Sennerby-Forsse 1985
Sycamore	4	64	0.412[2]	0.34-0.48	0.78NS	Webb et al. 1973
	4	64	0.40	-	1.10NS	Huber & Bongarten 1981
	7	21	0.460	0.444-0.480	0.87(0.73)NS[4]	Nebgen & Lowe 1981
	6	64	0.45	-	0.73(0.68)NS[4]	Land et al. 1983
	8	30	0.412[2]	-	0.55(0.50)NS[4]	McCutchan 1983
	5	48	0.445[2]	0.420-0.447	-	Jourdain & Olson 1984
Sweetgum	5	8	-	0.400-0.457	-	Makoui 1981
	10	8	-	0.423-0.487	-	" "
Black Locust	11	8	0.658	0.636-0.673	0.07BS	Whitmore 1973
	20	9	0.58	0.55-0.59	0.10BS	Stringer et al. 1982

Eucalyptus	7-12	25	0.568Kg/m^3	0.519-0.626	-	Zobel et al. 1982
	1.4	20	0.376g/cc	0.339-0.393	0.83(0.80)NS[4]	Wang et al. 1984
	13mo	27	0.377g/cc	0.341-0.418	-	Rockwood et al. 1988
	90mo	"	0.418g/cc	0.374-0.449	-	" "
	103 mo	"	0.422g/cc	0.396-0.464	-	" "
Hybrid	2	10	0.405	0.335-0.441	-	Anon. 1982
Poplar	10	5	0.477	0.368-0.419	-	Phelps et al. 1982
	4	9	0.29	0.21-0.36	-	Phelps et al. 1982
	3	3	0.32[3](0.40)	-	-	Phelps et al. 1987
Sycamore	6	64	0.44	-	0.29(0.14)NS[4]	Land et al. 1983
Eucalyptus	1.4	20	0.258g/cc	0.215-0.302	-	Wang et al. 1984

[1]BS = Broadsense & NS = Narrow Sense Heritability.
[2]Values are for wood with bark attached.
[3]First and coppice rotations, respectively.
[4]Family & individual tree values, respectively.

Significant variation in wood density occurred among the ten clones evaluated at two years of age. The overall average was 0.362; clone means ranged from 0.323 to 0.396. The average of the five clones also measured at age ten was 0.362, with means varying from 0.328 to 0.408. Ranks were similar at both ages. As an example, the clone with the highest wood density at 2 years was also highest at age ten. Early selection may, therefore, be a reliable means for hastening genetic improvement when rotations are 10 years or less. Considerably more variation within clones, however, was found at the younger age.

Differences among clones in terms of bark specific gravity were significant, but neither as large nor as frequent as those for wood. Overall average at age two was 0.405, and clone means varied from 0.355 to 0.435. The five clones measured at both ages had similar ranks. Clones with high or low wood density seldom ranked the same in terms of bark density.

Einspahr et al. (1967) evaluated extent of genetic variability in and control of specific gravity in and control of specific gravity in full-sibs of 5-year-old aspen (Populus tremuloides). The average across families was 0.37, with family means ranging from 0.34 to 0.40. Despite this seemingly small range, narrow sense heritability was estimated at 0.42. Weak correlation was found between specific gravity and growth. Prospects for improvement were considered favorable. In subsequent breeding work, selected native aspen were crossed with a tetraploid Populus tremula to give triploid hybrids (Einspahr 1984). Wood specific gravity at 15 years of age averaged at least 15 percent greater

than that of native diploids of both similar and greater ages. In addition, growth rates were doubled, and substantial improvements were obtained in fiber length and in several pulp and paper properties. Creation of polyploids may be useful for improving this trait and others in species used for energy production.

Olson et al. (1984) measured wood specific gravity of 3-year-old trees from 75 Populus deltoides clones growing in Kentucky. Results provided firm evidence for strong genetic control of specific gravity, and for negative relationships between volume growth and specific gravity as well as alpha-cellulose content (Table II). The authors concluded that opportunities for improving specific gravity were significant, but that some gain in volume growth would be sacrificed. Viewed another way, however, direct selection for volume was not expected to cause major reductions in specific gravity or alpha-cellulose content. Since only one location was involved, genotype X environment interactions were not assessed.

Working with nine clones of hybrid poplars, Phelps et al.(1982) evaluated specific gravity of wood and bark from 4-year-old trees. Clones of Aigerios-Tacamahaca parentage generally had higher stem and branch wood specific gravities than those from Tacamahaca parents. Similar differences were found for bark, but variation within the Aigerios-Tacamahaca group was also significant. Specific gravities of both stem wood and bark typically exceed those of branches, but the pattern of variation was inconsistent across clones. Unlike findings for sycamore (Platanus occidentalis) (Huber and Bongarten 1981), significant genetic variation was not noted for the quantity of vessel elements. Variation among the three planting densities was not significant for any of the measured variables. The authors concluded that genetic improvement of specific gravity was feasible, but that interactions among tissues and organs should be considered if whole-tree chips were used as feedstock.

In a related study with three of the same clones, Phelps et al. (1987) examined wood and bark specific gravity of 3-year-old trees from a first and coppice rotation. Trees from the coppice rotation grew faster and larger, and significant differences were found between bark and wood and among heights within trees. These and minor differences among clones, however, were not considered meaningful from a manufacturing standpoint.

Nepveu et al. (1978) determined basic wood density (wood without bark) of 1-year-old stems from 23 clones of Populus nigra and 28 clones of P. X Euramericana. Clonal differences were significant, regardless of parentage. Genetic correlations between densities of 1-year-old material and those of mature trees were also significant, (P. nigra = 0.72 and P. X Euramericana = 0.60) indicating good prospects for early selection. The authors also interpreted absence of meaningful correlations between density and growth to mean that selection for density would have no detrimental effects on growth.

TABLE II: Correlations Between Specific Gravity and Other Traits, Part A=Genetic Correlations.

Species	Trait	Correlation[1]	Author and Year
Eastern Cottonwood	Holocellulose	0.74	Olson et al. 1984
	Alpha-Cellulose	0.54	
Sycamore	Root Collar Diameter	-0.15	Webb et al. 1973
	Height, 4 years	-0.12	
	Basal Diameter, 4 years	-0.25	
	Percent Dry Wt., 4 years	-0.21	
Sycamore	Fiber Length	0.06	Huber and Bongarten 1981
	Percent Rag Volume	0.50	
	Percent Vessel Volume	-0.87	
	Percent Fiber Volume	0.52	
Sycamore	Height	0.28	McCutchan 1983
	Diameter	0.31	
	Predicted Dry Weight	0.28	
Sycamore	Stem Bark Sp. Gr.	0.42	Land et al. 1983
	Percent Moisture Content	0.00	
	Volume	-0.01	
Eastern Cottonwood	Basal Area	-0.172	Herpka 1979
Eastern Cottonwood	Alpha-Cellulose	0.34*	Olson et al. 1984
	Holocellulose	0.25*	
	Volume	-0.28*	
Aspen	Growth	-0.094*	Yanchuck et al. 1984
Sweetgum	Fiber Length	0.034	Makoui 1981
	Double Cell Wall Thickness	0.237*	
	Lumen Diameter	-0.482*	
	Fiber Diameter	-0.300*	
Sycamore	Root Collar Diameter	0.08	Webb et al. 1973
	Height, 4 years	0.30	
	Basal Diameter, 4 years	0.30	
	Percent Dry Weight, 4 years	0.28	
Sycamore	Height	0.12*	Jourdain & Olson 1984
	Diameter	0.23*	
	Volume	0.18*	

Footnotes: [1]Statistically significant values are marked with an *. All others are not significant.

Yanchuk et al. (1984) evaluated wood density (g/cc) of 15 putative aspen clones in Alberta. Trees were at least 36 years old, and samples, each four annual rings wide, were taken across the radii. "Clonal" variation was significant, and moderate broad sense heritabilities were noted for both density and fiber length (Table I). Wood density was high near the pith, decreased at short distances from the pith, and then increased to the bark. A small but negative genetic correlation was found between density and growth. The authors concluded that wood density could be improved, and that the negative relationship with growth could be offset by selecting clones with both fast growth and high density.

Nebgen and Lowe (1982) evaluated inheritance of specific gravity and other traits of 7-year-old trees from 21 open-pollinated sycamore families growing at two locations in Texas and one in Louisiana. Family and individual tree heritabilities were sizeable (Table I). Significant genotype X environment interactions were noted for growth traits, but not for specific gravity.

Land et al. (1983) estimated specific gravity (g/cc) of 6-year-old trees from four progeny tests of 64 open-pollinated sycamore families. Specific gravity of both wood and bark decreased from the base to the top of individual trees. Stem bark specific gravity was greater than that for wood at large stem diameters, but lower at smaller diameters. Family and individual tree heritabilities were sizeable (Table I), but larger for wood than for bark specific gravity. The genetic correlation between specific gravity and volume was negligible. Stem wood specific gravity had a positive correlation with stem bark specific gravity, but was not related to moisture content of wood and bark. Differences among sites were significant, with those producing the best growth also having lower specific gravities. Genotype X environment interactions, however, were not significant.

Generally similar and complementary estimates of genetic parameters (Table I) were found for young sycamore families by Jourdain and Olson (1984) and McCutchan (1983). Materials were grown at single locations, and genotype X environment interactions could not be estimated. Correlations between specific gravity and growth were small but positive.

Specific gravity, fiber length, and fiber, vessel, and ray volumes were measured at DBH in the second growth ring of coppiced sycamore from a progeny test in Georgia (Huber and Bongarten 1981). The trees had been established as 1-0 seedlings, and the shoots harvested at the end of the fourth growing season. Results were derived from coppice shoots harvested 2 years later. Significant variation was apparent for all measured variables, with that for tissue volumes being larger than that for specific gravity and fiber length.Specific gravity and fiber length, however, had higher heritabilities. Genetic correlations of specific gravity with ray and fiber volumes were positive and strong, whereas that with vessel volume was strong and negative. The relationship with fiber length was negligible. The authors concluded that selection for higher specific gravity was feasible, but that such action would also alter tissue volumes. Such correlated responses could be useful or detrimental, depending upon their effects on end use. Raising specific gravity can increase pulp yields, but elevated proportions of ray cells could reduce paper strength. While such trade-offs are understood, to some extent, for pulp and paper properties, they are not clear for energy conversion efficiency or

product yields. Effects of decreased vessel proportions on growth may also warrant investigation.

Specific gravity and several other properties of 10-year-old sweetgum (Liquidambar styraciflua) were assessed by Makoui (1981). Trees represented a sample of ten open-pollinated families from a South Carolina progeny test. Significant variation was found within and among families. Individual tree values averaged 0.45, and varied from 0.40 to 0.52. Family means ranged from 0.423 to 0.487. Specific gravity increased an average of six percent from the fifth to the tenth year, with percentage increases varying from 0 to 11 percent among families. Correlations among wood properties were computed for a selected subset of families, spanning the range of specific gravities. The correlation between specific gravity and cell wall thickness was positive and significant, while that with lumen diameter was negative and significant. Those with fiber length and diameter were not significant. Such results suggest that specific gravity is, in part, a function of variation in cell wall thickness. No information was provided, however, on variation in, or relationships with, volumes of various tissue and cell types.

Variations within trees, vertically and radially, were documented for European black alder (Alnus glutinosa) by Vurdu and Bensend (1979). Stem wood specific gravity in two 8-year-old specimens averaged 0.428, and increased only slightly with increasing height and distance from the pith. Similar trends for 17-year-old trees from a provenance trial in Ohio were noted by Robison and Mize (1987). Average specific gravity over all 13 provenances for wood from the first nine annual rings was 0.39. Provenance means ranged from 0.37 to 0.42, but variation among them was not significant. Individual tree means varied from 0.34 to 0.45.

Kenney et al. (In press) surveyed reports concerning specific gravity in Populus and Salix species. From reports not cited above, they found much the same type of data and drew similar conclusions.

In sum, greater specific gravity or density of wood can contribute much toward raising biomass yield, increasing energy content, and reducing conversion cost. Variation is abundant, and heritabilities are moderate to high across the species surveyed above. Some inconsistencies are apparent with regard to correlations among traits, especially growth rate. In general, however, specific gravity can be raised in hardwoods without detrimental effects on productivity. General absence of significant genotype X environment interactions should also simplify selection, breeding, and deployment. Some sensitivity to age was noted, but data on juvenile/mature correlations, though limited, suggest genuine opportunities for early selection, given the short rotations favored for energy production. Thus, specific gravity can be manipulated by selection and breeding with relative ease, and should be considered for integration with other traits in selection indices that optimize energy content and process efficiency. Efforts should include ways to increase uniformity as well (Wilcox and Smith 1973, Zobel et al. 1982). Availability of information is such that efforts can be initiated with a minimum of further research.

Additional work, nevertheless, is needed to discern what changes as specific gravity is altered. Evidence given above suggests that relative tissue proportions differed

in sycamore, that vessel size rather than proportion differed in poplar hybrids, and that cell wall thickness differed in sweetgum. Data is needed on what changes to expect, whether changes will vary among species and how such changes will affect utility for energy production and other uses. Savidge (1986) contended that changes associated with and causes of increased specific gravity in existing tree improvement programs are not well understood. They may have resulted from formation of more latewood, more uniform cell wall thickness, or yet other factors. He suggests increasing specific gravity by reducing lignin content and holding cell wall thickness constant. Since cellulose has higher specific gravity than lignin, this approach would raise specific gravity, and perhaps yield more useful wood. Perhaps this is one way that molecular genetics can be used to influence a useful but complex trait. Regardless, efforts to alter specific gravity should be prefaced by research on underlying causes, the anatomical and chemical changes that occur, and the effects such changes will have on suitability for intended uses.

MOISTURE CONTENT

Moisture content significantly affects conversion efficiency, especially for thermal conversion and direct combustion. Costs and time associated with drying vary directly with moisture content. Regardless of process, however, the greater weight of high moisture content feedstocks affects harvesting, handling, and transport costs. Lower moisture content, obtained via genetic manipulation, timing of harvest, or other means is desired.

Wood and bark moisture content have been investigated in several species. Somegenetic variation is apparent, but results are far from clearcut. Such traits tend to vary within trees, and are affected by season and year. Anderson and Zsuffa (1982) nevertheless describe an example in which small but significant variation occurred among hybrid poplar clones.

Moisture content of wood and bark from 2- and 10-year-old trees of five hybrid poplar clones was analyzed by Anon. (1982). Wood moisture content varied with height, but no consistent pattern was evident. Moisture contents of wood from older trees were almost twice those of younger materials. Significant clonal differences were observed, with means for younger wood ranging from 69 to 87 percent, and those for older wood varying from 135 to 176 percent. Bark moisture also varied with height, being greatest at the top. Unlike wood, however, bark moisture content was greater in young trees. Clonal differences were significant for bark, and generally larger than for wood. Rankings of wood and bark values for
older and younger trees generally agreed, suggesting early selection for this trait may be possible. Some representative clone means are:

Age and Tissue Type	% Moisture Content	
	High	Low
2 Yrs		
Wood	87	69

	Bark	157	108
10 Yrs			
	Wood	176	135
	Bark	94	80

Land et al. (1983) evaluated moisture contents (wt./unit vol.) of wood with intact bark of trees from 64 open-pollinated families of sycamore. Four parents from each of 16 seed sources were represented. Trees were 6 years old and growing at four locations in Mississippi. Moisture contents were greatest at the tops of individual trees. Site differences were significant, with moisture contents lowest on the best sites. Seed source and family effects, genetic correlations, and genotype X environment interactions, however, were not significant, and the authors concluded that moisture content could not be altered by selection and breeding for it or any other measured trait.

In contrast, Jourdain and Olson (1984) found significant differences in moisture 48 open-pollinated sycamore families (5 years old) at a single Kentucky location. Moisture content of individual trees averaged 113 percent, and ranged from 90 to 134 percent. Family means ranged from 103 to 122 percent. Moisture content was correlated negatively with diameter (-0.13) and specific gravity (-0.57). Genetic modification of moisture content was considered possible, but only to a minor extent.

McCutchan (1983) assessed variation in moisture content of trees in an 8-year-old open-pollinated trial of sycamore growing in North Carolina. Stem moisture content averaged 138 percent. Family and individual tree heritabilities were estimated at 0.64 and 0.89, respectively. Genetic correlations (not significant) with moisture content were calculated for height = 0.11, diameter = 0.14, and predicted tree dry weight = -0.08. Results were used to assess effectiveness of indirect selection for tree dry weight, and effects of such selection on other traits. Tree dry weight was correlated most strongly with diameter, height, and crown length. Given the various heritabilities, genetic correlations, and practical considerations, height was considered the best trait for use in improving overall tree dry weight. The positive correlation between height and moisture content would cause somewhat higher moisture content, but the authors considered an overall increase in dry weight more important, and advocated offsetting the increased moisture content by harvesting season and technique as suggested by Land et al. (1983). Using diameter as the selection criterion would give similar improvement in dry weight without adversely affecting moisture content. The authors recommended height, however, as selection for diameter would give less improvement in crown length and configuration, live crown ratio, and straightness.

Schultz and Land (1987) examined feasibility of early selection in sycamore. Genetic and product moment correlations were computed for five seed traits, two nursery trials, four early field traits, and fifth year diameter, height, stem + branch dry weight, and volume. Field data were derived from the four progeny tests described above (Land et al. 1983). Significant product moment correlations with dry weight were found for germination value (0.305), root collar diameter after the first growing season

(0.565), and several other field traits. Essentially the same relationships were found between fifth year volume and these seed and early field traits. As a result, germination value and first-year root collar diameter were recommended for use in early selection.

Somewhat higher values for percent moisture content and heritability were reported for Eucalyptus grandis by Wang et al. (1984). The 1.4-year-old trees were from 20 open-pollinated families growing in south Florida. Percent moisture contents were determined separately for wood and bark. Family means for wood ranged from 142 to 166 percent, and for bark from 226 to 341 percent. Values for bark moisture content exceeded those for wood as was noted above for young poplar hybrids (Anon. 1982). Differences within and among families were significant, indicating substantial genetic variation. Family and individual tree heritabilities for wood moisture were estimated at 0.60 and 0.28, respectively. No correlation existed between moisture content and specific gravity. The authors indicated that selection for wood moisture content was warranted, and was unlikely to alter specific gravity. They, however, lacked sufficient confidence in their bark moisture findings to estimate heritability.

Similar means, ranges, and correlations were given for a wider array of Eucalyptus families and species in a later summary. (Rockwood et al. 1988). Results are not detailed here as additional heritability values were not listed. The authors noted, however, that moisture contents of older and coppice trees were lower than those for young trees. They again asserted that moisture content is under modest genetic control.

Conclusions from the review by Kenney et al. (In press) generally mirror those summarized above; no additional heritabilities were given.

Reports cited above thus suggest that moisture content is under some degree of genetic control, and not strongly correlated to other traits. Magnitude of heritability values is encouraging, despite some inconsistency. Not to be overlooked, however, are the sizeable and variable influences of age, tissue type, location within tree, and site. Large effects caused by season and year can also be expected. Under these circumstances, attempts to modify moisture content via conventional or other genetic means seem best prefaced by further research. Perhaps some needed information on genetic and environmental influences can be accumulated in quick order and at low cost by judicious analysis of extant or future "energy plantations."

BARK QUANTITY

Trends in harvesting, handling, and processing woody plant biomass are such that bark most likely will not be removed prior to processing. Since bark physical and chemical properties differ from those of wood, variation in bark quantity and/or quality can affect process efficiencies. The following considers opportunities for manipulating bark quantities; information concerning chemical composition, e.g., lignin, are considered in other sections.

In discussing variation in calorific values among Populus hybrids, Sastry and Anderson (1980) indicate that factors underlying clonal differences are not clearly understood, and that bark quantities may be more critical than chemical constituents such as extractives. They further indicate that bark amounts vary inversely with tree diameter, and that both growth rate and clonal effects may influence the contribution of bark to energy content.

In commenting on these findings, Anderson and Zsuffa (1982) suggest that calorific differences are related to bark content in Populus, and clonal variation in bark content approximates that for lignin and other constituents.

Significant variation among eastern cottonwood provenances, families, and individual trees was noted by Cheng-Chun (1974) for a variety of traits. Bark thickness was measured at DBH on all trees 30 ft or taller. Thickness was correlated with height (0.68) and diameter (0.75), but not bark texture. Trees from Indiana and Ohio sources had thick bark, whereas bark of Nebraska, Kansas, and South Dakota sources was thinner. Trees representing Minnesota, Missouri, Illinois, and Mississippi sources were intermediate.

Bark thickness varied inversely from the base to the top of 2-year-old hybrid poplar clones. Bark nevertheless comprised a larger proportion of total volume and weight in samples from the tops (Anon. 1982). In some cases, bark from tops amounted to 50 percent of sample weight. Variation among the ten clones was significant. The two most productive clones had consistently low proportions of bark.

Phelps et al. (1982) evaluated bark percents of 4-year-old trees of nine hybrid poplar clones growing in northern Wisconsin. Bark quantities were higher for branches than stems. Clonal differences were significant, but variation was minor (range = 27 to 34 percent). The several planting densities had no effect on bark percent.

Bark percentages of 1-year-old shoots from 20 clones, representing four Salix species and hybrids were determined by Sennerby-Forsse (1985). Samples were collected in each of three consecutive growing seasons. Clonal effects were significant; means ranged from 24.9 percent to 37.9 percent. Clones having low wood specific gravity tended to have the highest bark percents. Four clones, all from a single taxon - S. dasyclados var. aquatic Gigantea, showed exceptional stability over growing seasons. Bark percents for most others varied considerably among growing seasons.

Stem plus limb bark volume averaged 11 percent of green volume per tree in four 6-year-old sycamore progeny tests (Land et al. 1983). Sixteen open-pollinated families from each of four seed sources were represented. Family means ranged from 4 to 13 percent. Estimates of heritabilities and other genetic parameters were not given.

Makoui (1981) evaluated bark, wood, and pulping properties of trees from eight open-pollinated families of sweetgum. The families represented a sample drawn from a 10-year-old progeny test growing in South Carolina. Representative values are:

Bark Trait	Mean	Range
Percent/vol.	23.01	12.89-32.43
Percent/wt.	10.79	7.42-24.84

Both family and individual tree variation were significant. Although other genetic parameters were not listed, the author suggested that selection and breeding would lead to improvement in these and other wood properties of sweetgum.

The review by Kenney et al. (In press) contains findings much the same as those above. Data from the above sampling of reports indicate that bark thickness and proportion are much affected by position within tree, tree size, and age. Biomass harvested from small stems, e.g., generally contains large proportions of bark. Some reports also suggested that bark characteristics vary with site and growth rate but not spacing. Despite the influence of such factors, clonal and family ranges in bark thickness and bark percent are substantial for several species, especially when materials other than the older poplar hybrids are considered. Heritabilities and other genetic parameters nevertheless were not found, and reliable estimates should be gathered before practical improvement is attempted. Given the findings to date, however, efforts to seek modification seem warranted. One possible strategy might involve choosing an optimal tree size and bark/wood ratio, and then breeding to adjust bark content in the direction desired for process needs, provided that potential interactions with growth rate and harvest size are considered. In addition, potential effects on tolerance of biotic and abiotic stresses must also be evaluated.

EXTRACTIVES

Hardwood extractives primarily consist of fatty acids, esters, and non-saponifiable materials, and mainly occur in parenchyma cells (Browning 1963). Amounts typically are small in comparison to other constituents, but are sufficient to cause problems in processing. In pulping and papermaking, e.g., extractives consume extra chemicals, discolor pulps, form pitch deposits, and corrode equipment. Similar difficulties as well as disposal problems may occur in ethanol production. In these instances, lower extractives content or altered composition may be desirable. On the other hand, constituents such as fatty acids, alcohols, and esters contribute to energy content, and larger amounts of high energy forms may be preferred in thermal conversion.

Extractives soluble in alcohol-benzene from various Populus species and hybrids were assayed by Einspahr et al. (1968). Mean contents varied from 2.1 to 6.3 percent, but the authors considered the spread rather narrow in view of the number and variety of taxa.

In contrast, the same authors found moderate to good genetic control of extractives content in a full-sib test of 5-year-old aspen (P. tremuloides). Contents averaged 4.1 percent, and ranged from 3.0 to 5.3 percent. Despite this seemingly small range, heritability was estimated at 0.87. The authors cautioned that the heritability, computed from interclass correlations, was probably an "upper limit."

Comparable variability was found by Yanchuk et al. (1987) among 13 mature aspen clones. Extractives were most abundant near the pith (6.1 percent) and decreased to a low nearest the bark (3.5 percent). Clonal differences were significant, and means varied from 3.5 to 4.9 percent. Broad sense heritability was estimated at 0.13, dramatically lower than the narrow sense value noted above. Phenotypic and genetic correlations between growth rate and extractive content of the first eight annual rings (that stem portion most representative of energy crop rotations) were - 0.191 and -0.452, respectively.

Dickson et al. (1974) examined extractives from the third annual ring of 18 clones from a variety of intra- and interspecific Populus hybrids. Contents averaged 3.3 percent, ranged from 2.2 to 4.1 percent, and were somewhat lower than but still in relative agreement with values noted above.

Anderson and Zsuffa (1982) cite an example in which bark extractives of willow clones ranged from 27 to 40 percent.

Extractives in wood, bark, and wood/bark composites from seven hybrid poplar clones were assayed by Blankenhorn et al. (1985a). Differences among clones generally were significant, but tissue type and age effects were larger. In samples of 8-year-old material, e.g., clone means ranged from 5.00 to 6.28 percent for wood and from 7.94 to 10.16 percent for wood/bark composites, whereas those for bark ranged from 22.69 to 31.90 percent. Age effects generally were significant, and contents tended to decrease with age. The authors concluded that clonal effects were important, but tissue types (wood vs. bark) and age were more so.

In a separate report concerning the same clones, Bowersox et al. (1979) found that wood/bark samples from 4-year-old trees had extractives contents ranging from 7.4 to 17.8 percent, thus confirming the tendency for contents to decline with age.

Blankenhorn et al. (1985b) examined wood, bark, and wood/bark samples from three of the same clones grown on two different sites. They confirmed that younger materials contain more extractives, and showed that extractives content varied with rotation. Significant clonal differences were apparent only for the oldest materials (4 years old). Site effects were also noted, but were variable across tissue types. Wood and wood/bark extractives levels were highest in trees from the lower quality site, whereas bark extractives were greatest in trees from the better site.

Alcohol-benzene and water soluble extractives of 11-year-old black locust clones were assessed by Whitmore (1973). Average alcohol-benzene extractives content for the eight clones was 4.55 percent, with a range of 3.32 to 5.409 percent. Water soluble

extractives averaged 5.99, and varied from 5.08 to 7.48 percent. Broad sense heritabilities were 0.54 and 0.05, respectively. The 0.54 value for alcohol-benzene extractives exceeded those for all other traits, including specific gravity.

Arguing that extractives were a major contributor to energy content, Stringer et al. (1982) assessed variation in a 20-year-old trial of nine black locust clones. Differences among clones in terms of hexane and alcohol soluble extractives were significant. In contrast, hot water and total extractives did not vary significantly among clones.

Means, ranges, and broad sense heritabilities are summarized below.

Extractive Category	Mean	Range	Heritability (Broad Sense)
	-% o.d. wt.-		
Hexane	0.28	0.12-0.53	0.48
Alcohol	3.34	2.33-3.81	0.23
Hot Water	4.00	3.29-4.59	0.06
Total	7.62	6.88-8.66	0.07

The heritability values for alcohol and water soluble extractives shown above are lower than those reported by Whitmore (1973), but are of the same relative order. Thus, both reports suggest that at least extractives soluble in neutral organic solvents can be manipulated via conventional breeding.

Similar but slightly lower means were reported by Stringer and Olson (1987) for ten 10- to 12-year-old black locusts growing on two sites. They also documented trends in radial and vertical contents of extractives within trees. Radial variation was significant for some extractives. Sapwood contained the lowest benzene-alcohol (2.7 percent) and total extractives content (6.81 percent), while outer heartwood had the highest (4.60 and 8.54 respectively). Content also varied with height; hot water soluble extractives increased while benzene-alcohol extractives decreased. Site effects were not apparent.

Some additional means and ranges for poplars and willows are provided by Kenney et al. (In press). In general, these are comparable to those discussed above. No information beyond that summarized above was given on genetic parameters. Reports described above generally indicate that extractive contents are similar within species and among related species. Large differences, however, are apparent within individual trees, between tissue types, e.g., wood vs. bark, and even among ages. Some hint of site effects was also noted. Such observations infer that environmental and other

influences are substantially greater than slim, and heritabilities are low to moderate. Most authors, however, were optimistic about manipulating extractive contents via conventional breeding. Improvement or modification most assuredly could be made, but progress would be slow. Given the paucity of information, further and more reliable estimates of heritability, genetic correlations, and genotype X environment interactions are needed. In addition, few data are available on composition of extractives and genetic variation therein. Certain components, e.g., fatty acids, have high energy contents, and primarily function as storage compounds. Selectively manipulating them could have useful outcomes without harming productivity. Phenolic components, however, have been implicated in pest resistance, and direct or indirect changes in them could prove harmful. Clearly, better understanding of the composition and role of extractives as well as their quantities is needed. Conventional selection and breeding can be used to untangle such mysteries, and molecular genetics can perhaps be bent to that end as well.

ASH CONTENT

Ash contents of woody plant biomass typically are low, and not considered of great importance in either biological or thermochemical conversion processes. Impact on site nutrient status, transport costs, and pollution or disposal problems, however, should be considered. Some possibility also exists that certain metallic elements could harm catalysts or foul equipment used in thermochemical conversion. Though not all that critical, lessening amounts could be helpful, mainly for thermal conversion processes and from the stand-point of preventing inordinate site nutrient drain.

Ash contents of most North American woods typically range from 0.2 to 0.9 percent, and are often less than 0.5 percent (Browning 1963). Distribution is uniform throughout the wood. Composition varies, but usually consists of 40 to 70 percent calcium oxide, 10 to 30 percent potassium oxide, 5 to 10 percent magnesium oxide, and 0.5 to 2.0 percent ferric oxide. Other metals are represented in smaller amounts.

Literature on genetic variation in ash content is sparse, and findings are variable. Murphy et al. (1979) evaluated ash contents of three hybrid poplar clones grown at five planting densities. Stem wood sections from 4-year-old trees were analyzed. Ash content averaged only 0.5 percent. Neither clone nor spacing effects were significant, and no clone X spacing interaction was apparent.

In contrast, Anderson and Zsuffa (1982) describe Populus clones containing half as much ash as others, implying that clones could be developed that have low ash contents and greater nutrient use efficiency.

Analyses of six hybrid poplar clones in Ontario highlight differences between wood and bark (Anon. 1982). Samples were taken from the base, midstem, and branches of one-to 3-year-old trees. Bark ash content (4.9 percent) exceeded that of wood (0.9 percent). Contents in wood increased from the base to branches, while that in bark decreased. Clonal effects were small, and significant only for bark.

Bowersox et al. (1979) examined ash and macronutrient contents of 4-year-old trees of seven hybrid poplar clones. Ash in composite wood/bark samples averaged 0.80 over all clones, and ranged from 0.60 to 1.20 percent. Clonal differences were not significant. Some clones, however, differed in their P, Ca, and Mg contents, but no consistent relationship to parentage was noted. Assays from different places within trees of one clone confirmed that bark contained more ash and macronutrients than wood/bark composites. The authors concluded that repeated harvests could measurably drain site nutrients, and that clonal effects may influence product yields and nutrient status.

Evidence of clonal variation was also found by Blankenhorn et al. (1985a). Analyses were performed on wood, bark, and wood/bark samples representing tissues from one- to 8-year-old trees. Ash contents of bark were more than four times those of wood. Age effects were significant and content increased notably with age. Comparisons among the seven clones at 8 years, an age desirable for efficient harvesting, showed significant differences for ash contents of wood, bark, and wood/bark samples. Ranges were: wood = 0.76 to 1.27 percent, wood/bark = 1.54 to 2.03, and bark = 6.02 to 7.48. Such differences suggest some potential for altering ash content, especially that of bark. Regrettably, however, ash content of wood did not appear related to that of bark. Indeed, the clone with the least ash in wood had the greatest amount in bark. Lastly, clonal differences were less than those for age and tissue types.

Reports cited above indicate that ash content of bark is more important than that of wood. They also provide some, though conflicting, evidence for genetic control over ash and macronutrient content, and for altering these characteristics via selection and breeding. Modifying bark seems more feasible and productive. Such opportunities deserve closer examination, particularly as concerns lessening ash content of bark and reducing bark/wood ratios. Before attempting change, however, heritabilities, genetic correlations, and genotype X environment interactions should be discerned. Data on interactions are critical in that mineral content of pulping liquors and associated scaling of pulp mill boilers and evaporators varies greatly among regions. Problems are particularly serious in areas having calcareous soils.

CALORIFIC VALUE

Direct combustion is a major means of extracting energy from woody plant biomass, and calorific value is often used as an index of fuel quality. This characteristic, however, is not necessarily a good indicator of value for other conversion processes. Ethanol production, e.g., varies with carbohydrate content, and a particular sample of biomass may derive its high calorific value from large concentrations of extractives.

Nevertheless, genetic variation in calorific value has been observed, and several authors have examined the influence of various environmental and management factors. Small but significant variation among hybrid poplar clones was reported by Sastry and Anderson (1980), Anderson and Zsuffa (1983), and Blankenhorn et al. (1985a). In most

instances, however, the extent of variation was small. Also, Bowersox et al. (1979) and Anon. (1982) found no clonal differences among hybrid poplars. In contrast, broad sense heritability was estimated at 0.68 in black locust by Stringer et al. (1982). Age seemed to have no effect on calorific value (Blankenhorn et al. 1985a), and tissue type had little effect (Bowersox et al. 1979). Wood had greater calorific value than bark in young hybrid poplar (Anon. 1982), but the opposite was found in other work with similar hybrids (Blankenhorn et al. 1985a). Sastry and Anderson (1980) found substantial variability among 2-year-old poplar hybrids, but no apparent association with specific gravity. In contrast, Anderson and Zsuffa (1982) suggested an association between calorific value and extractive content.

In their review of Populus and Salix, Kenney et al. (In press) concluded that variability was slight, and that genetic gain was likely to be minor despite moderate broad sense heritabilities.

Suffice it to say that the picture for calorific value is confusing, and that utility of this composite trait from the standpoint of biological and thermochemical conversion is rather limited. In addition, modifying such a complicated trait without understanding the contribution of its components seems dangerous. Selection and breeding for calorific value could cause unknown changes in contributing factors and substances, perhaps leading to biomass of limited value or ill suited for other uses/products.

SUITABILITY FOR OTHER USES

Past research on "energy plantations" has shown that biomass productivity of useful species can be raised dramatically by breeding and intensive culture. Growth is rapid, and rotations are reduced to 12 years or less. Many such species have utility for other uses, especially pulp and paper manufacture. Suitability for one or more uses in addition to energy production would give growers more flexibility and help attract investment. Understanding how past increases in productivity and future changes in physical and chemical characteristics affect suitability for other uses and markets is therefore an important issue.

Several traits discussed elsewhere in this report, e.g., specific gravity and cellulose content among others, significantly affect pulp and paper manufacture. Generally, many of the changes in quantity and quality described herein would seem beneficial. Even the correlated responses from direct selection and breeding for such traits do not seem detrimental.

Fiber properties, particularly fiber length, also deserve consideration. Einspahr et al. (1967) found significant variation in fiber length among full sib aspen families. They found a modest value for heritability (Table III), and strong correlation between fiber length and growth. Efforts to improve aspen resulted in creation of triploid hybrids that had fiber lengths at least nine percent greater than those of native populations (Einspahr 1984). Several paper properties were also enhanced.

TABLE III. Means, Ranges, & Heritabilities for Fiber Length.

Species	Age (Yrs.)	Genetic Entries (#)	Mean (mm)	Range (mm)	h^2 [1]	Author and Year
Aspen	5	25	0.62	0.55-0.69	0.58NS	Einspahr et al., 1967
Aspen	36	15	-	0.67-0.99	0.93BS	Yanchuck et al., 1984
Sweetgum	10	8	1.60	1.57-1.62	-	Makoui 1981
Sycamore	5	48	1.54	1.45-1.67	-	Jourdain & Olson, 1984

[1]BS = Broad Sense & NS = Narrow Sense Heritability.

Yanchuk et al. (1984) also found a relationship between fiber length and growth in aspen. Though not significant, the genetic correlation between fiber length and growth was 0.577. They also indicated that clones with longer fibers at early ages also had greater fiber lengths as mature trees. Such relationships indicate that selection for increased growth is likely to improve fiber length, and that early selection may be possible.

In their analysis of 4-year-old poplar hybrids, Phelps et al. (1982) noted that fiber length varied significantly among clones, but was not affected by planting density. In a later test involving three of the same clones, fiber lengths at three years of age did not differ meaningfully between trees from a first and coppice rotation.

Significant genetic variation in fiber length of 5-year-old sycamore families was reported by Jourdain and Olson (1984). They also noted significant correlations between it and height, diameter, and volume. The correlation with specific gravity was not significant.

Makoui (1981) examined several fiber characteristics in sweetgum. Trees were 10 years old, and represented eight families from an open-pollinated progeny test in South Carolina. Some degree of genetic variation was found for fiber length, double cell wall thickness, lumen diameter, and fiber diameter. Correlations of fiber length with

the other fiber dimensions were not significant. Variation in pulp yields was not significant. Comparison of wood and pulp properties from these materials to those of 30- to 56-year-old trees showed that young trees had slightly higher, though nonsignificant, values for moisture content, specific gravity, pulp yield, and several paper properties. The author concluded that young and old trees produced pulps with similar physical properties.

Barker (1974) evaluated wood, pulp, and paper properties of young green ash, sycamore, water oak, and sweetgum trees. Direct comparisons were made between young and more mature trees for sweetgum and oak. Representative values for species of greatest interest from the standpoint of energy production are excerpted in Table IV. The author concluded that young hardwoods were comparable to mature trees in terms of pulp yields and chemical demands for pulping and bleaching. With few exceptions, wood, pulp, and paper properties were also similar. Similar conclusions were reached by Jett and Zobel (1975) and Zobel (1981).

Despite the foregoing observations, those responsible for manipulating species and populations for increased energy production and conversion efficiency should maintain large breeding populations and preserve flexibility in breeding programs. With such provisions, the abbreviated rotations, relatively short generation intervals, and amenability of useful species to asexual propagation should foster progress and allow for changes in direction. With time, advances in molecular genetics should permit using the same base material for different purposes, and also provide for rapid changes in direction.

RECOMMENDATIONS

Opportunities for manipulating traits of interest via conventional selection and breeding vary considerably. Priorities for manipulation or research also vary with potential impact of a trait on process efficiency.

Cellulose and lignin quantity and quality rank highest in terms of benefit. For cellulose content, the range of variation is quite narrow, and genetic control seems weak and nonadditive in nature. Uncertainties exist with regard to nature and strength of correlations with other traits. Even so, improvement in cellulose content has been achieved in some species, and overall prospects appear promising, provided that efficient methods of vegetative propagation are available for capturing nonadditive variation. Future research should concentrate on acquiring better estimates of genetic parameters, especially relationships with other traits. More and better data on correlations could lead to improvement via indirect selection. Further attention to methods for mass clonal propagation is also warranted. Research on cellulose structure, its variability, and effects on conversion efficiency are also needed.

Opportunities for manipulating lignin content via selection and breeding appear greater than for cellulose. The range of genetic variation is small but useful. Despite variability in size and accuracy, heritabilities are somewhat larger than those for

TABLE IV. Some Properties of Young Southern Hardwoods.[1]

Property	Green Ash (9 yr.)	Sycamore (11 yr.)	Sweetgum 13 year	Mature
Sp Gr	0.60	0.42	0.47	0.48
% Glucans[2]	44	45	41	43
% Lignin	25	21	20	21
% Extractives	1.4	0.8	1.5	2.0
Bleached Yield, %	47	48	48	48
Bulk, cm^3/g^3	2.1	1.9	1.8	1.8
Breaking Length, km	2.3	3.9	4.7	4.0
Stretch, %	2.8	3.1	4.8	2.6
TEA, $ft\text{-}lb/ft^2$	4.8	5.2	16.2	3.8
Tear Factor	50	101	128	95
Burst Factor	32	58	83	53
Scattering Coeff., cm/g	423	397	297	312

[1]Adapted from Barker, 1974.
[2]% Glucan = Indicator of Cellulose Content.
[3]All paper properties evaluated after 10 min of refining.

cellulose. Thus, exploratory work to alter lignin content appears fruitful and can be attempted now at least on a trial basis. Additional information on genetic parameters, accumulated in the process, could be used to evaluate feasibility of larger, more earnest efforts. A better understanding of lignin quality, particularly methoxy content, is also needed. Such research could be executed in conjunction with work on manipulation via molecular genetics.

Specific gravity and moisture content are also important in producing fuels from biomass. Higher specific gravity and lower moisture content would improve efficiencies of harvesting, transport, handling, and conversion. Genetic variation in specific gravity is large, and heritabilities are moderate to strong. Moreover, early selection seems possible, and could hasten progress. Incorporation into existing breeding programs should be worthwhile, provided impact on other traits and overall breeding efficiencies are monitored. Simultaneously, research should be done to document causes of increased specific gravity; i.e., underlying changes in cell wall thickness, proportions of cell types, and/or chemical composition. Effects of changes on process efficiency should be monitored as well.

Moisture content is under some degree of genetic control, and heritabilities are moderate though variable. Correlations with other traits are unfavorable, but not strong.

Available information, however, does not present a clear sense of the ease and cost with which meaningful improvement can be obtained. Despite moderate heritability, the trait is influenced greatly by age, tissue type, location within tree, and site. Season and year can also have strong effects. Given these circumstances, breeding for lower moisture content should be considered, but is best prefaced by further gathering of information on genetic and environmental control.

Bark quantity seems of only modest importance. This may be fortunate in that little information on genetic variation or control was obtained. Available data suggests that manipulation of bark/wood ratios deserves some consideration, but only after further information has been accumulated and evaluated. Bark/wood ratios vary greatly with tree age and size. Thus, decisions about tree size and an acceptable ratio should be made before choosing breeding strategies. Potential trade-offs in terms of reduced resistance or tolerance to pests and stresses should also be clarified.

Extractive contents are low in comparison to other constituents, and changes therein seem far less important. Increased concentrations of fatty acids and other high energy compounds may be the only components worth altering. Several authors, nevertheless, reported heritabilities of modest size, and advocated breeding to alter extractive content. On the whole, however, data on genetic variation, inheritance patterns, and correlations with other traits were not abundant or convincing. Data on altering composition as opposed to quantity were particularly scarce. Much research is needed before serious attempts at selection and breeding are considered.

Of the traits in question, ash content seems least important. Genetic variation is minor, and little evidence of genetic control exists. Since ash varies more in bark than wood, modifying bark ash content or bark quantity may be more fruitful. In any case, much more information is needed before breeding can be justified. Future research should give special attention to site effects and genotype X environment interactions. Soil nutrient status has profound effects on elemental composition of wood and bark.

Increasing calorific value seems inappropriate. Simply stated, raising calorific values would not necessarily increase quantity or quality of constituents important to a particular process. Indeed, the biomass in question could be rendered less desirable for that process and/or other uses.

Much effort has been devoted to increasing growth and yield of "energy plantation," and impressive growth rates have been obtained on initial and coppice rotations of 12 or fewer years. Knowledge of how these improvements and future changes in physical and chemical properties affect other uses deserves more attention. In general, few effects detrimental to pulp and paper production are apparent or anticipated. Indeed, wood from young, fast-growing trees is acceptable for making many products, and is somewhat better than that from older trees for some grades of paper. This is not to say, however, that all future changes will be neutral or beneficial. Hence, suitability for other uses should be monitored as changes in strategy are implemented. In addition, traditional breeding efforts should be structured to maintain large breeding populations across time. Pursuit of a specific objective, e.g., low lignin content and high

specific gravity, seems best done with a subgroup of materials. Keeping original breeding populations separate and intact will provide flexibility to change directions and pursue differing objectives in subsequent rotations. Such an approach will also ensure continued availability of material for manipulation via molecular genetics.

BIOSYNTHETIC PATHWAYS: BASES FOR MANIPULATION BY MOLECULAR GENETICS

The enzymatic conversion of lignocellulosic biomass into biofuel is an important aspect of total wood utilization (Khol'kin, 1982; Shimizu et al., 1983). Principally, this means the enzymatic conversion of the cellulose portion to glucose (saccharification) which is then fermented to ethanol although hemicelluloses might also be saccharified and fermented to biofuel (Yu et al., 1984; Yu et al., 1985; Dalessandro and Northcote, 1977). For this process, lignin is considered to be not only unusable [although lignin could yield methanol (Ander and Eriksson, 1985)] but in the way of efficient utilization (Katkevich et al., 1985). Consequently, genetic improvement of woody feedstocks for this process should involve lowering of lignin content relative to that of polysaccharides. In the course of investigating this area, it has become apparent that any genetic modification that would allow easier removal of lignin might also be of considerable or even greater practical value. The other side of the coin would be to seek an increase in lignin and/or extractives content for the nonbiological conversion processes (Brown, 1985; Trenina, 1986; Ochi et al., 1984; Jodai, 1987). Some are of the opinion that bioconversion is not going to be successful (Sarkanen, 1985). Still others seek to use lignin residues from the bioconversion processes for specialized nonfuel products (Muller and Glasser, 1984).

METABOLIC PATHWAYS

Any effort to interdict lignin biosynthesis may have some undesirable side effects on tree growth and development, but this is a consideration for most genetic modifications since our knowledge is still very limited in this respect. If one starts with glucose, whether it arises from photosynthesis directly or via storage forms like starch or transport forms like sucrose, the biosynthesis of lignin is a rather long pathway. Two major pathways of carbohydrate metabolism, glycolysis and the pentose phosphate pathway, give rise to two essential starting materials, phosphoenolpyruvic acid (PEP) and erythrose-4-phosphate (E-4-P), respectively. These two phosphate derivatives are combined in the shikimic acid pathway to eventually yield an aromatic ring in the form of phenylpyruvic acid which can be converted to the aromatic amino acid, phenylalanine (Figures 1 and 2).

Except for a minor route (probably not operating in trees) via another aromatic amino acid, tyrosine, practically all of the carbon found in lignin ultimately comes from phenylalanine via the enzyme, phenylalanine ammonia lyase (PAL). Enzymatic steps forming a series of phenolic acids beginning with trans-cinnamic acid (the immediate

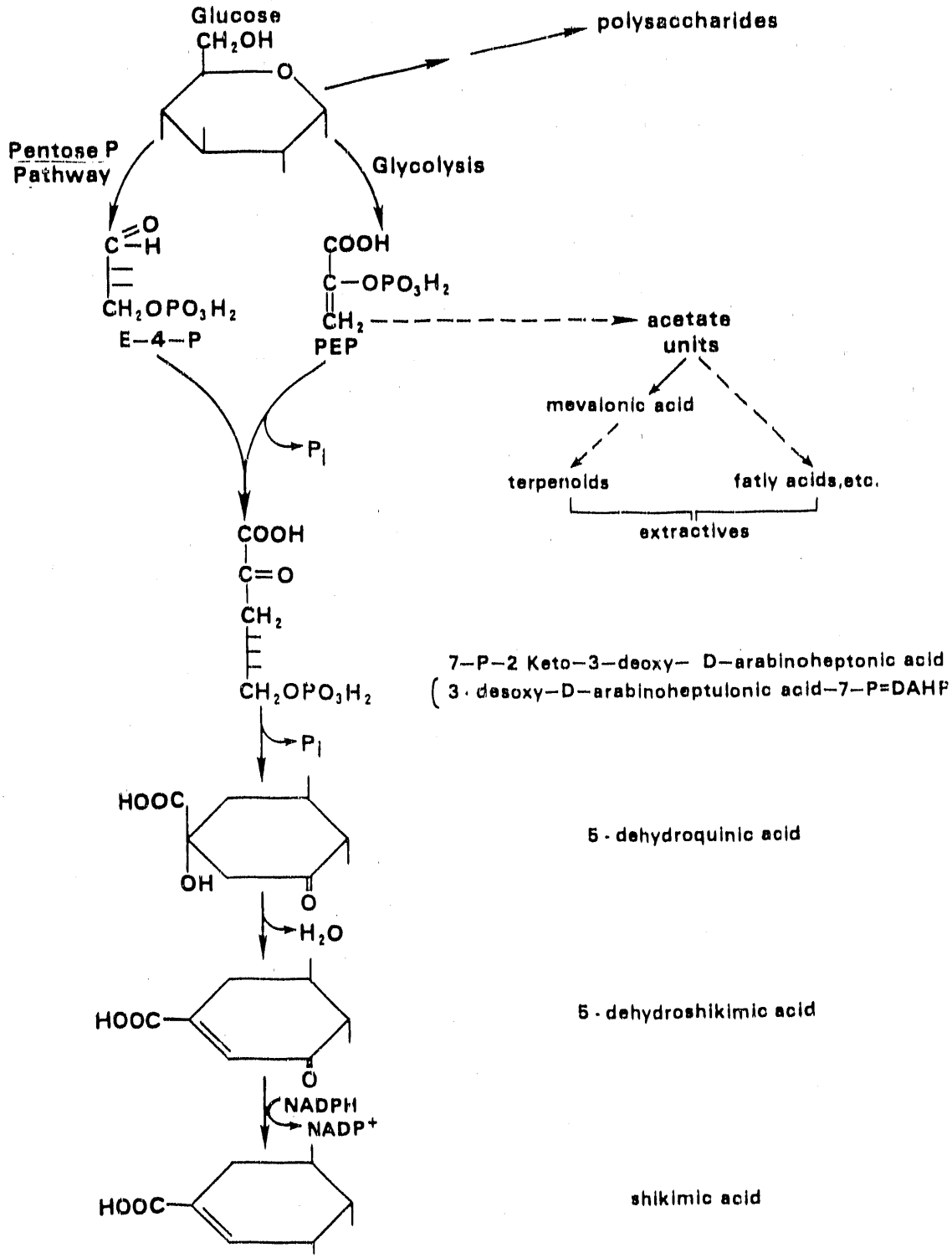

Figure 1. Origins of shikimic acid.

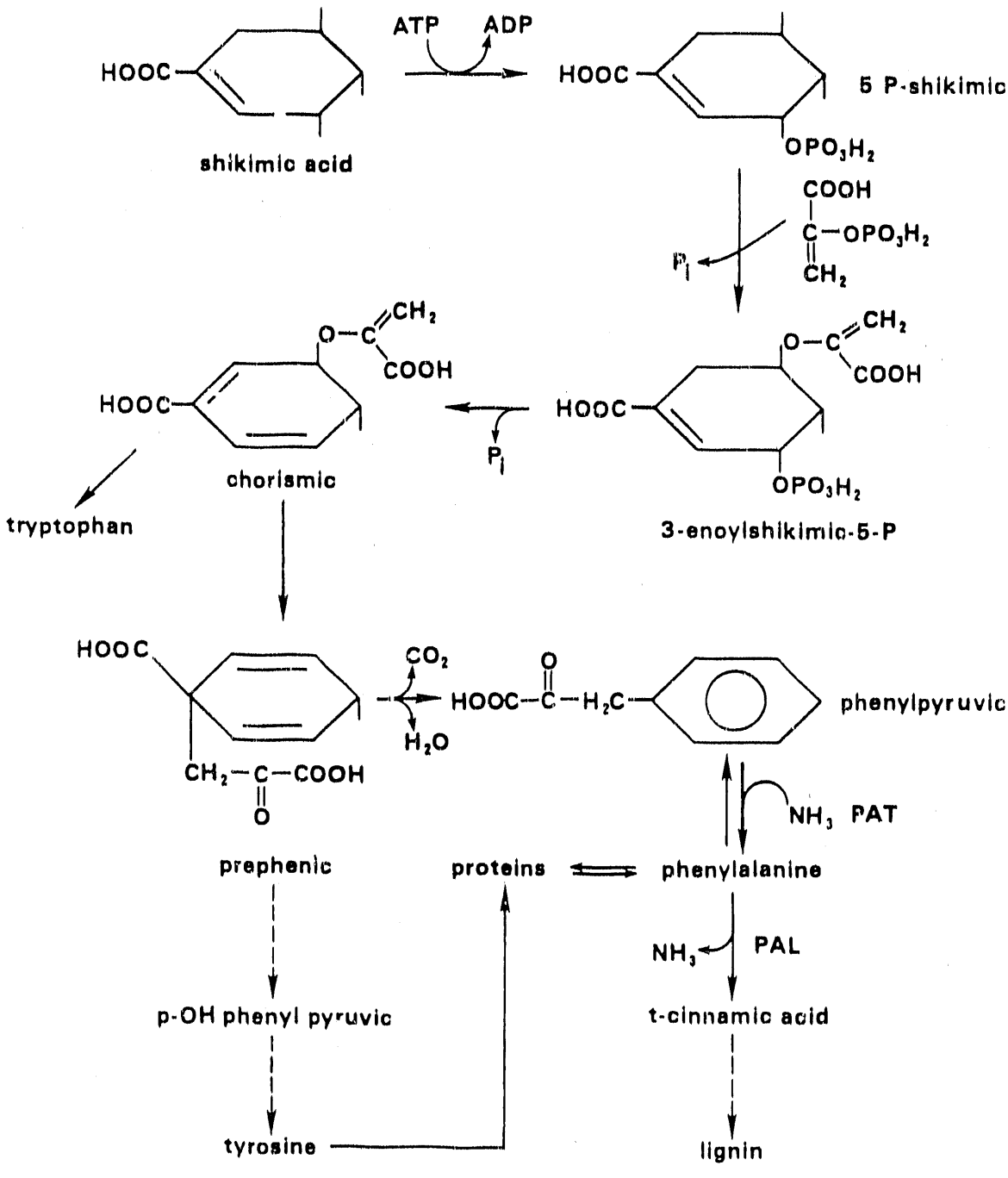

Figure 2. Shikimic to cinnamic acid.

product of PAL action) precede the formation of the lignin polymer. These steps involve hydroxylation and methylation reactions, the latter apparently committing these precursors to lignin biosynthesis as opposed to other possibilities, principally flavonoids and tannins. The anabolism of lignin commencing with PAL action is depicted in Figure 3. Peroxidase is shown as the enzyme initiating the random polymerization of the precursors; however, some investigators may still consider laccase for this role and recently questions have been raised about lignin's long held status as a "random polymer" (Renganathan et al., 1986; Agarwal and Atalla, 1986). Here, and in other figures, such as the metabolism of cell wall polysaccharides (Figure 4), not only the metabolites are shown, but also the known biochemical reagents and cofactors because they may provide little recognized or ignored control possibilities. Enzymes catalyzing the various steps are given in many instances since they would be major targets in any attempts at genetic control.

Cell wall composition and biosynthesis have been reviewed frequently (Bolwell, 1988; Gross, 1985, Bacic et al., 1988; Delmer and Stone, 1988; Dey and Brinson, 1984; Grisebach, 1981; Haigler, 1985; Higuchi, 1985; Hori and Elbein, 1985; Hillis, 1985; James et al., 1985). Since the cell wall is not a simple entity, the interplay of biosyntheses and control are complex. In addition to genetic control, many spatial and temporal factors influence the product. Consequently, development phase, light, wounding, attack, etc., are all influential.

It should be appreciated at the outset that the biosynthesis mechanism is not completely clear in the case of any of the major wall polymers (cellulose, hemicelluloses celluloses, and lignin). Generally speaking, the uncertainty increases as the final polymerization event approaches.

SPECIFIC ENZYMES AND OTHER PARAMETERS

Attempts to solubilize, purify and characterize cellulose synthase and any other polysaccharide synthases have met with difficulties. Many of these membrane-bound proteins are probably glycoproteins; therefore, not only would genetic control need identification of the gene coding for the enzyme, but also of correct glycosylation patterns. Cellulose synthase itself has never been identified to the satisfaction of the scientific peer group, although several solubilized preparations have been put forth for this role over the past 25 years (Delmer, 1983; Roberts, 1984; Delmer and Stone, 1988). Usually, the rates at which they make beta-1,4 glucan linkages are feeble or there is confusion about the substrate or product. In the latter cases, callose of some kind of callose-cellulose hybrid often seems to result. As Delmer (Delmer, 1987; Delmer and Stone, 1988) has noted, there have been significant advances on this front in recent years. A cyclic guanine dinucleotide was found to activate cellulose biosynthesis in Acetobacter xylinum but not in plants. Also, a receptor protein for an herbicide that blocks cellulose biosynthesis in plants may turn out to be a controller guaranteeing the specific cellulose product, perhaps including orientation in the wall and crystallinity.

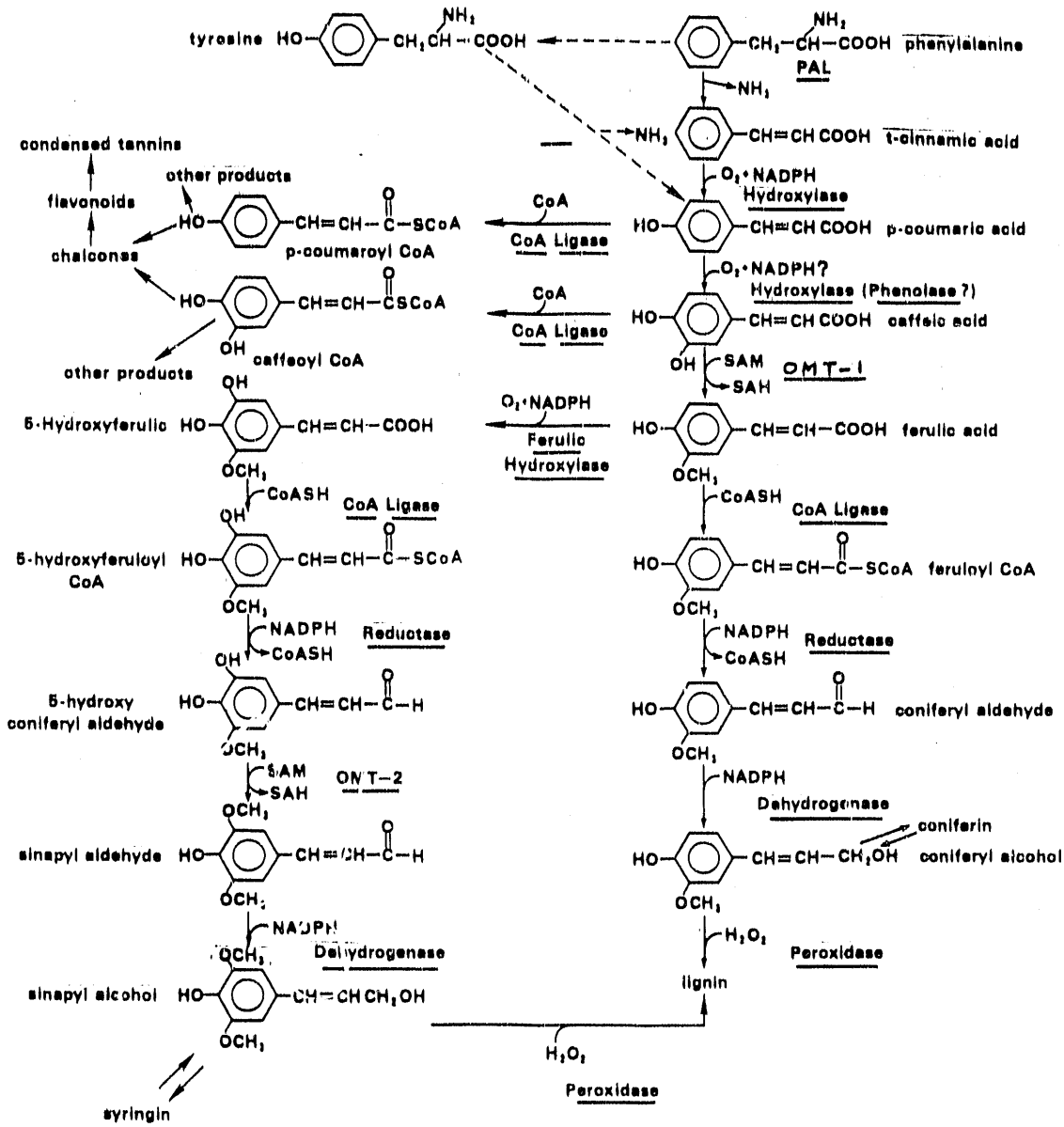

Figure 3. Phenylalanine to secondary products.

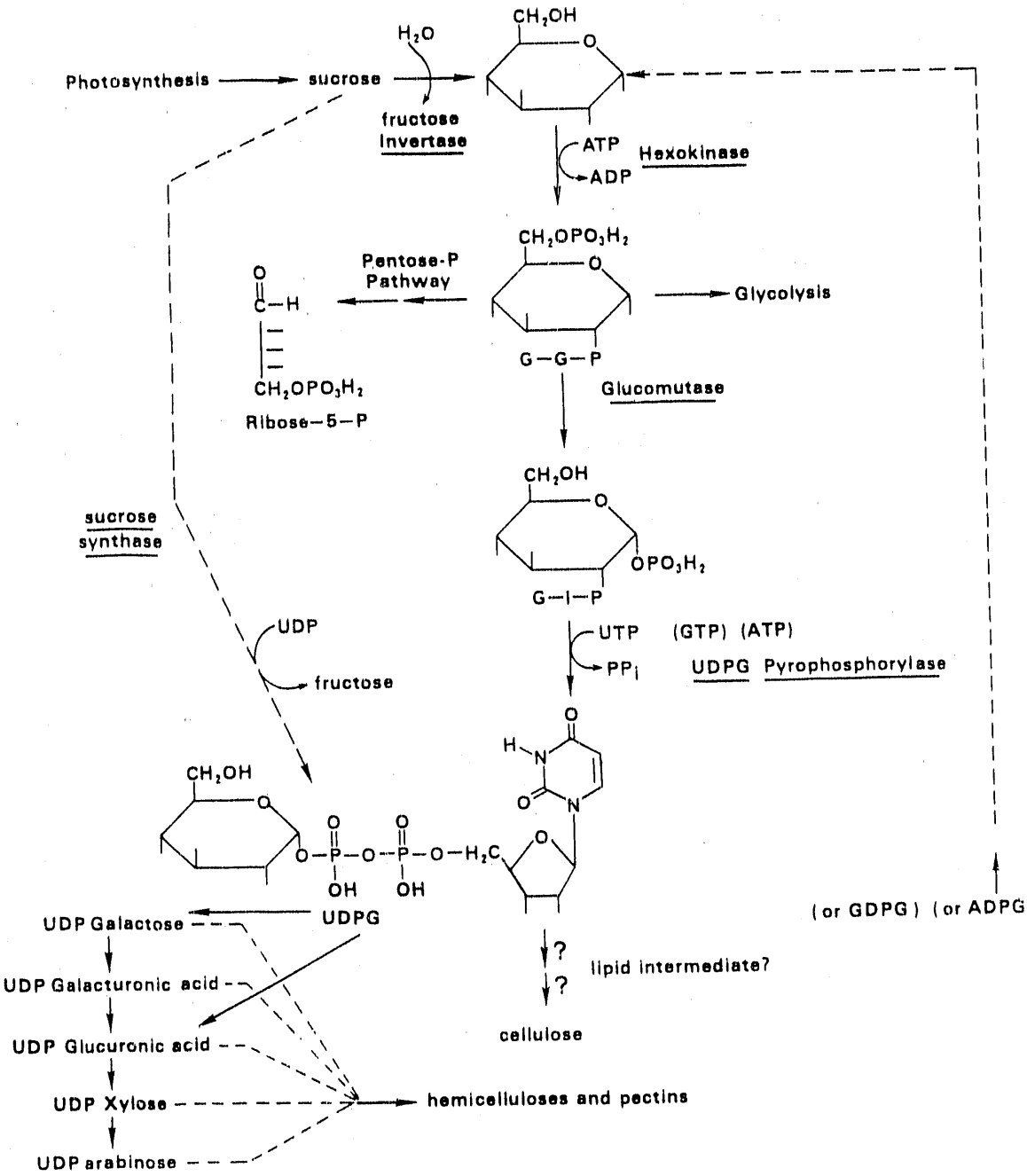

Figure 4. Carbohydrate metabolism.

Biosyntheses of hemicelluloses and pectic substances are often complicated by sequence and proper branching considerations. Unlike the template mechanisms operative for nucleic acids and proteins, concerted synthesis by multienzyme complexes or multifunctional enzymes appears the rule (Dalessandro et al., 1988; Delmer and Stone, 1988). Numerous glycosylating enzymes with varying specificities are being investigated.

One might expect that the capacity to form specific nucleoside diphosphate sugars, e.g., UDPglucose, GDPglucose, UDPxylose, etc., could be exploited to control polysaccharide products. However, to tap this area would require not only knowledge of the biosynthetic steps but also of the enzymes involved in the many possible interconversions of nucleoside diphosphate sugars and derivatives thereof (Hori and Elbein, 1985). Data of this type are still skimpy for given systems, especially trees, but includes feedback inhibition of UDPG dehydrogenase by UDPxylose and modulation of xylan synthase activity by nucleoside diphosphates and monophosphates (Dalessandro and Northcote, 1977). Specific hydrolases involved in the loosening of the cell wall during synthesis might provide a point of intervention in the synthesis of wall polysaccharides. Structural protein in the cell wall might also be subject to manipulation, but disruption at the primary wall synthesis stage might well be too far-reaching.

When it is desired to control lignin biosynthesis, much attention is often focused on phenylalanine ammonia lyase (PAL), for as already noted, practically all lignin carbon must have passed through this funnel (Hanson and Havir, 1981; Jones, 1984; Delmer and Stone, 1988). Considerable research effort has been expended on PAL preparations from several sources. cDNA's now exist for bean and parsley varieties (Edwards et al., 1985; Kuhn et al., 1984). A list of cloned genes of phenylpropanoid metabolism in plants was published recently by Mol et al. (1988); intentions are to update it periodically. All PAL molecules studied have been tetramers with a molecular weight over 300,000 daltons and a high pH optimum approaching 9 (Harborne, 1980). Despite this emphasis on PAL in the literature, it may be somewhat misplaced [see discussion of PAT (phenylalanine aminotransferase) in regulation below].

Hydroxylase enzymes catalyzing the formation of p-coumaric, caffeic and 5-OH ferulic acids are not yet highly purified, but they are cytochrome P450 mixed function oxidases (Potts et al., 1974; Rich and Lamb, 1977; Grand, 1984), with the possible exception of the one acting on p-coumaric acid (Mayer, 1987). Among the possibilities for this step, phenolases plus ascorbic acid may be used (Harborne, 1980). The O-methyltransferases (OMT's) of lignin biosynthesis occur in several different forms (e.g., Hermann et al., 1987; Poulton, 1981) and are different from those of flavonoid biosynthesis (Poulton, 1977; Poulton et al., 1976; Kuroda, 1981). In hardwoods the lignin OMT is difunctional and methylates not only caffeic acid to ferulic acid but also forms the additional methoxyl groups found in the syringyl moieties of hardwood lignins. These enzymes require S-adenosyl methionine (SAM) as the methyl donor. Conceivably, genetic control of SAM biosynthesis could affect the formation of lignin precursors; however, SAM has other vital functions so allowance for some SAM synthesis would be necessary (e.g., Bachrach, 1973).

Like SAM, coenzyme A (CoA) availability should also influence lignification. However, its alternate functions are even more widespread than those of SAM. It is also regenerated in subsequent steps whereas SAM is converted to SAH (S-adenosylhomocysteine). Compartmentalization always enters as well into any considerations to render cofactors like these as limiting upon a specific pathway, i.e., lignin biosynthesis in this case. As can be seen in Figure 3, enzymes forming CoA esters (CoA ligases) come into play en route to both tannins and lignin (Knobloch and Hahlbrock, 1975; Knobloch and Hahlbrock, 1977; Heinzmann and Seitz, 1977; Grand et al., 1983). Some gene sequencing is in hand for these enzymes; at least one has been purified from a tree source (spruce) (Luderitz and Grisebach, 1981). Energy in the form of ATP is needed to form the CoA esters (Hahlbrock and Grisebach, 1979). Unfortunately, there have been some indications that these enzymes are either very transient or unstable in hardwoods, at least in extracts (Gross, 19 5). They are also subject to light modulation (Feutry and Letouze, 1984).

Enzymes called chalcone synthases are also of some interest in the context of this discussion since they could compete for p-coumaroyl and caffeoyl moieties (as CoA esters) that could otherwise wind up in lignin. The chalcone synthases provide for the entrance of this carbon into flavonoid and condensed tannin biosynthesis routes (Harborne and Mabry, 1982). Some other competing enzymes are also possible, but they should be of minor importance in the systems under consideration here and will not be discussed.

Many of the enzymes of lignin biosynthesis mentioned so far have been shown to exist as isozymes (Hanson and Havir, 1981) and subject to feedback and feedforward regulation as well as induction and repression. Cinnamic acid is a particularly busy molecule in this respect, relating to both PAL and chalcone synthases. Among other things, cinnamic acid promotes the synthesis of another protein which modifies the PAL active site causing inactivation of PAL (Bolwell et al., 1986). An endogenous macromolecular inhibitor of PAL has been observed too (Harborne, 1980). Biosynthesis of PAL is influenced not only by cinnamic acid but also by light. Cinnamic effects supposedly occur at the translation stage whereas light affects transcription (Lamb, 1979), but this is not without exception (Bolwell et al., 1988). Much of the foregoing may require reevaluation in light of the unappreciated importance of PAT noted earlier.

Da Cunha, (1987) showed that many of the common assays used for PAL are not specific enough and measure combined PAL + PAT activity. Given properties of these two enzymes, much of PAL activity appearing in the literature could really be reporting mostly PAT activity (in the direction of phenylalanine to phenylpyruvic acid), "mostly" meaning a PAT/PAL ratio approaching ten-fold. Therefore, reported effects of PAL may have been at best indirect through action on PAT. Obviously, PAT and protein synthesis, both of which compete for phenylalanine, need more attention relative to control of phenylpropanoid biosynthesis via PAL. The conclusion of Da Cunha and others (Harborne, 1980) that phenylalanine supply is the major controlling factor has considerable merit.

The immediate low molecular weight precursors of hardwood lignin are the alcohols formed by reduction of CoA esters of two phenylpropanoids, i.e., feruloyl CoA and sinapoyl CoA. p-Coumaroyl CoA may play a minor role; it is significant only in the formation of grass lignins. Most authors consider these to be two-stage processes with the intermediate formation of the corresponding aldehyde. cDNA's for enzymes catalyzing this type of reaction have been obtained from bean and poplar (Bolwell, 1988; Mol et al., 1988). Although both guaiacyl and syringyl lignin are deposited in hardwoods, much is not formed simultaneously nor in the same locale. Terashima et al. (1986) report that most syringyl lignin is formed late and mostly on fiber rather than vessel walls. Both synthesis (Lewis et al., 1987; Shann and Blum, 1987; Lewis et al., 1987) and analysis (Saka and Goring, 1988; Eom et al., 1987) approaches are used in this area. The action of wall peroxidase isozymes leads to the formation of phenoxy free radicals from the foregoing alcohols (coniferyl and sinapyl, respectively) (Imberty et al., 1985). These radicals may react with each other to form lignin, or they may react with other wall components to form cross links, so-called lignin-carbohydrate bonds (discussed later). Not only are isoperoxidases required at this point (Stich and Ebermann, 1988) but also hydrogen peroxide, and possibly other cofactors involved in its formation (Goldberg et al., 1985; Gross, 1985; Renganathan et al., 1986). Cross linkages involving isotyrosine and ferulic dimerization appear mainly a feature of the primary wall with unknown repercussions on overall wall structure (Fry, 1986a).

Temporal gene expression during wall biosynthesis is, of course, subject to many controls (Savidge, 1988; Delmer and Stone, 1988), particularly the plant hormones whose real mechanisms of action are yet unknown. Thus, control of hormone concentrations should in turn affect various aspects of wall biosynthesis. For example, auxin-induced cell elongation results in increases in both xyloglucan and cellulose synthase activities as well as in beta-glucanase activity. The latter is thought to act mainly on xyloglucan leading to oligosaccharin formation. The oligosaccharin then acts like an antiauxin resulting in some spectacular morphogenic responses (Fry, 1986b).

As seen in Figures 1-4, there are many events separating polysaccharide biosynthesis from lignin biosynthesis. Connections to terpenoid extractives (Hillis, 1985, Croteau and Johnson, 1985) are even more obscure since sugar carbons would have to be converted to acetate units which would then enter the mevalonic acid pathway. In hardwoods the extractives are mostly fatty acids, esters and other nonsaponifiable lipids (Yanchuk et al., 1988; Roschin et al., 1986; Ohara et al., 1986). These other nonphenolic extractives also form from acetate units via the mevalonic acid or other pathways. Because the carbohydrates could serve as precursors to both lignin and extractives, promoting polysaccharide biosyntheses should eventually elicit a response of less lignin and extractive biosynthesis due to competition for building blocks. Conversely, lignin and extractives are very unlikely starting materials for carbohydrate biosyntheses, so no competive interactions are expected. On the other hand, mutual effects upon specific steps are quite likely among these three major synthesis routes (Mason and Wasserman, 1987; Savidge, 1987). Synthetic inhibitors tors of, e.g., specific steps in lignin biosynthesis as well as in the biosyntheses of other cell wall components, have been tested also (Grand et all, 1985; Delmer and Stone, 1988).

Since lignification is a late event in cell wall formation and since lignin is a major stumbling block in the utilization of lignocellulosics by enzymes to form ethanol, efforts to control this process would seem to hold some of the best promise. Laboratories at Michigan Technological University (Chiang et al.) and North Carolina State University (Sederoff et al.) are mounting sizable efforts to control lignin biosynthesis for pulping purposes (e.g., see Williams, 1988). These efforts indicate that enough is now known about the biosynthesis of lignin that it should also draw considerable attention relative to biofuels, whether to lower lignin content or modify it for bioconversion or to increase lignin content for nonbiological conversion processes.

There is some evidence that coniferyl and sinapyl alcohols, substrates for peroxidase-catalyzed lignin formation, must be transported as glycosides (coniferin and syringin) from formation at the endoplasmic reticulum to the cell wall polymerization site (Freudenberg and Harkin, 1963; Gross, 1985). Upon arrival at the wall, glycosidase action would be necessary before peroxidase action could begin.

Beta-glycosidases are common in cell wall preparations (Marcinowski and Grisebach, 1978). Formation of glycosides requires nucleoside diphosphate sugars (especially UDPG). Thus, both synthesis and cleavage of coniferin and syringin offer potential to favor or block lignification.

LIGNIN-CARBOHYDRATE BONDS

Lignin is generally recognized as the major impediment to saccharification of lignocellulosics by enzymatic means (Monties, 1985; Bezuch, 1988; Rivers, 1983; Husain, 1983; Feniksova et al., 1981; Katkevich et al., 1985). Much of this lignin may be simply "encrusted" on fibers, but some lignin covalent bonds to polysaccharides have been found before (Tanabe and Kobayashi, 1988; Joseleau and Kesraoul, 1986; Fukuda and Kohmoto, 1986; Yanilkin et al., 1987) and after pulping of lignocellulosics by various means (Takahashi and Koshijima, 1988; Erin'sh et al., 1982; Zakharov et al., 1982; Gerasimowicz et al., 1984; Watanabe et al., 1985; Iversen, 1985; Azuma et al., 1985). Whether these linkages exist in vivo is somewhat uncertain yet, but most investigators seem to accept this as fact. Other wall parameters like cellulose crystallinity (Takai, 1984; Karaushi, 1985; Prati et al., 1985; Levanova et al., 1981; Katkevich et al., 1985; deConinck-Chosson, 1988) can also be barriers to bioconversion.

PRETREATMENTS TO BIOCONVERSION

Many pretreatments of lignocellulosics are used to make the cellulose more accessible to attack by cellulases, either per se or as secreted by fungi and other organisms. Some, such as alkali treatment, only seem to make matters more difficult by smearing the lignin over the fibers and plugging lumens (Donaldson et al., 1988; Arai et al., 1985). Many others are beneficial. These include acid treatment (Ucar and Fengel, 1988), microwave and other irradiation (Magara et al., 1988; Klimentov, 1985), steam explosion (Donaldson et al., 1988; Yu and Saddler, 1987), hydrothermolysis (Hormeyer

et al., 1987; Hormeyer et al., 1988)), organic solvents (Hormeyer et al., 1987), etc. (Wei and Cheng, 1985; Wayman et al., 1987; Parekh et al., 1988).

Many additional authors have examined wood structure from the point of view of susceptibility to microbial or enzymatic degradation (Jeffries, 1987; Deshpande and Eriksson, 1984; Weimer and Weston, 1985; Daniel and Nilsson, 1985; Wiley, 1985; Yu et al., 1985; Faix et al., 1985). Cellulase genes have been cloned (Pasternak and Glick, 1987; Beguin, 1988). Some polysaccharidases are subject to inhibition by low molecular weight phenolics (Sharma et al., 1985), some of which might originate from cell wall degradation. A recent addition to the arsenal of wall-degrading enzymes is one from a bacterium, Erwinia spp. It is an arylетherase that has been cloned and used to transform Escherichia coli cells (Srinivasan and Cary, 1987). Other genomic libraries related to the degradation of lignin model compounds are being constructed (Katayama et al., 1988).

RECOMMENDATIONS

Outlook

Opportunities for specific genetic interference with polysaccharide biosynthesis would appear to be far off at present. The various enzymes involved in the fundamental polymerization processes are too poorly characterized to proceed by molecular biology methodology. It might become possible to interfere with secondary aspects such as branching before tampering with the primary process. Glycoside formation or breakdown is discussed later relative to lignin precursors and might be an interesting point for intervention.

Enhancing the levels of certain polysaccharides by preventing their degradation or lowering their levels by boosting the degradative endogenous hydrolases is also not attractive at this time. Those polysaccharidase activities, e.g., cellulase, endogenous to plants seem to be essential for the overall growth of the cell wall so that effects well beyond just preserving or destroying certain polysaccharides are apt to result. Interference at the level of structural cell wall protein is fraught with the same considerations. Likewise, tampering with cofactors such as the nucleoside diphosphate sugars would be a complex approach which still lacks enough firm data, particularly for tree species.

The terpenoid extractives are so far removed metabolically from the other lignocellulosics that it is doubtful that, for example, blocking their biosyntheses would have much effect on lignin, cellulose and hemicelluloses unless it were via a specific chemical interaction of which there seems to be little current knowledge. Ont he other hand, if one wanted to increase the content of these extractives for the nonbiological conversion processes, the wide metabolic separation from the other lignocellulosics could be advantageous. This is also true to a lesser extent for extractives like fatty acids which are more important in the angiosperms. All terpenoids originate from acetate units entering the mevalonic acid pathway, so the enzymes catalyzing the first condensations

there might be suitable points for intrusion. Fatty acids and related molecules also originate from acetate via multienzyme fatty acid synthesis complexes which are worked out for only a few biosystems. Dealing with extractives at the level of their degradation is a remote possibility; in that case the pathways for fatty acid breakdown have been known for quite some time, but terpenoid catabolism is not well studied, particularly in the woody angiosperms.

Enhancing or suppressing lignin biosynthesis without much effect on the other lignocellulosics probably holds the greatest promise at present. One reason for this is that lignin is considered to be a secondary product laid down late in the development of the cell wall. As such it may not have great repercussions should its content be raised or lowered or its structure modified. Of course, there are no guarantees for this, but relative to each of the other components it appears to be the safest bet. Furthermore, although there are some steps requiring further work the long pathway from sugar to lignin is known and a few genes for enzymes along the way have been cloned.

Because the PAL enzyme catalyzing the conversion of phenylalanine to cinnamic acid is considered to be the initial reaction in all secondary product formation, it is a logical place to attempt control. PAL genes from at least three plant sources are already available. There is the possible disadvantage that more than lignin should be affected by enhancing or suppressing PAL activity; however, as their secondary product designation suggests, these other products (flavonoids, tannins, phenolic acids, etc.) are usually considered to have attractive/repulsive functions which are more helpful than vital. There seems to be ample evidence that lignification and polysaccharide biosynthesis can proceed independently (e.g., Savidge, 1986).

PAL has always been an easy focal point because to go back any further in the pathway would be to interfere with the formation of the aromatic amino acids which are essential for the synthesis of many proteins. Nevertheless, as discussed earlier in conjunction with PAT activity, phenylalanine concentrations per se may be more critical than the amount of PAL enzyme at hand. For example, during periods of rapid growth, demand for phenylalanine for protein synthesis could preclude much flow through PAL since, at low phenylalanine levels, PAL is at a disadvantage versus both protein synthesis and PAT. More attention should be given to PAT enzymes with various kinetic properties. Hardly any effort has gone into PAT research to date. Both PAT and PAL seem to require considerable push or pull to function in the direction of secondary product synthesis.

The hydroxylase enzymes beyond PAL in the direction of lignin biosynthesis are resisting purification. There would appear to be no particular advantage in working with them unless one were trying to promote syringyl over guaiacyl lignin. In that case one would focus on the ferulic acid 5-hydroxylase and the 0-methyltransferase enzymes forming sinapic acid. The 0-methyltransferases catalyzing ferulic acid formation commit the benzene ring to lignin as opposed to other secondary product synthesis. The 0MT's are probably easier to work with than the hydroxylases whose manipulation could still be voided by direction of the resulting products into products other than lignin.

Although the enzymes (CoA ligases) forming CoA esters of the phenylpropanoic acids have been purified from plants, their dependence upon available ATP and CoA would be complicating factors in trying to manipulate their activities in vivo. Blocking activities of CoA ligases acting on p-coumaric and caffeic acids might prevent the drain of these precursors into other secondary products if one wished to promote lignin biosynthesis. Since some success in cloning enzymes of the cinnamyl alcohol dehydrogenase type has been forthcoming even for poplar, investment of some effort here might be rewarding. However, a cofactor requirement (reductant) could also present a problem in vivo in this case.

Some attention was given in the review to various cofactors associated with the many steps of lignin biosynthesis and how manipulation of their syntheses/degradation might be a point of attack. Manipulation of cofactors like NADPH, CoA, and SAM would seem to have too many ramifications beyond the target steps to be feasible in the foreseeable future. Controlling the hydrogen peroxide or some related molecule needed for the final polymerization of cinnamyl and/or sinapyl alcohols by peroxidase could have merit. Unfortunately, its mode of formation on site is still under investigation and fairly complex also. If the many peroxidases or isoperoxidases could be sorted out, some control over both qualitative and quantitative aspects of lignin biosynthesis might be possible. First, however, it will be necessary to establish which are real and which are artifacts, which are specific for lignin formation and particular lignin precursors, which are located in the wall, etc. for a given tree species.

Future Directions

As already stated, lignin appears to be the lignocellulosic most susceptible to and most promising for intervention, whether through conventional or molecular genetics. Regardless of the current state of the art, the following topics are worthy of investigation to gain control (positive or negative) over lignin biosynthesis: (1) manipulation of PAT activity, (2) control of coniferin/syringin metabolism, (3) control of hydrogen peroxide concentration in the cell wall, and (4) control over the extent or nature of lignin-carbohydrate bonding in the cell wall. Rationale for selecting these four areas is given below.

Control might be possible at the PAL/PAT/protein juncture. If CoA ligases for p-coumaric and caffeic acids were simultaneously controlled, one could also promote or prevent the drain of these precursors to other secondary products. However, were biofuels to be produced by nonbiological processes, these other secondary products might be just as valuable as lignin. PAL control alone may well have deleterious or even lethal consequences due to impact on protein synthesis. It seems unlikely that the control of any single enzyme beginning with PAL will get the job done despite the fact that most of the carbon in lignin originates from phenylalanine via PAL. PAT could be the real control point. If only small amounts of phenylalanine are provided via PAT, they will be used for needed protein synthesis. If large amounts of phenylalanine are formed via PAT, they will be acted upon by PAL without adverse effects on protein

biosynthesis. Other sources of phenylalanine are possible (protein degradation for example), but PAT should be by far the most important.

Transport and compartmentalization factors have been ignored in these recommendations up to this point. However, they are very real considerations incomplete systems. Consequently, formation, transport and breakdown of molecules like coniferin and syringin might prove vulnerable to control with significant impact on quality and quantity of lignin. More work would be necessary with the enzymes involved before any molecular approach could be attempted.

Lignin precursors could not be polymerized without hydrogen peroxide or some related molecule being available at the wall site. This might be a difficult subject for genetic control. Peroxidase control might also be useful, but these enzymes have been studied for years and still present a confusing picture.

No other enzymes between PAL and peroxidase offer great control advantages with exception of OMTs where the ferulic formers would commit precursors to lignin and sinapic formers would affect the nature of lignin subunits. Control of syringyl/guaiacyl rations might in turn influence extent of lignin-carbohydrate bounds. As mentioned above, the amount of this bonding between lignin and hemicelluloses could in turn affect possibilities, for example, of removing lignin from lignocellulosics to allow saccharification/fermentation of cellulose to ethanol. Whereas there have been numerous studies of these bonds, there has been relatively little investigation yet of their biosynthesis or biodegradation. More of the latter would be necessary to appreciate enzyme(s) in a given species before much could be done at the molecular level. However, one could consider conventional breeding for low or high lignin-carbohydrate bond content.

POTENTIAL APPLICATIONS OF MOLECULAR GENETICS

BACKGROUND, DEFINITIONS AND APPROACHES

Genes controlling physiological, anatomical and structural traits important to growth and end-use properties of trees can be captured and manipulated several ways. Historically, tree improvement programs have relied upon "tried and true" methods familiar to classical breeders. Typically, variation is measured in diverse populations; if the range of variation is sufficient, then individuals from the populations are crossed to capture gains afforded by genes in the "variant individual." Application of these techniques has been successful in improvement of both simply controlled traits (disease resistance) as well as those under polygenic control (quantitative traits). Progress has been significant and must be appreciated. Perhaps the future will prove these methods the most efficient and economical means to improve forest tree species. Progress is arduously slow, however, compared to the achievements with agronomic crops.

Biotechnology has generated interest among workers concerned with genetic improvement of forest trees. Mass propagation of elite genotypes through tissue culture techniques may be among the first applications. Faithful reproduction of desired genotypes can be difficult due to difficulties in vegetative propagation, lack of inbreds, and high degree of heterozygosity present in natural populations. Vegetative propagation of hardwoods has been carried out very successfully, this approach affording the substantial benefits of clonal propagation related to capture of nonadditive and epistatic gene interactions. Advantages of clonal propagation of forest trees have been the subject of several reports (Libby and Rauter, 1984; McKeand and Weir, 1984). Vegetative propagation can be carried out two ways: 1. through use of rooted cuttings or root sprouts (termed macropropagation) 2. through generation and multiplication of shoots from organs or tissues cultured in vitro (termed micropropagation). Economic analyses of such systems have been performed, estimating costs that could be tolerated and offset by increased performance of the improved stock (Hasnain, et al., 1986).

A second promising, though more speculative, aspect of biotechnology that may find use in forest tree improvement programs involves genetic engineering. Envisioned are programs in which the gene(s) controlling a desired trait are isolated, cloned into a suitable vector and introduced into the host plant. This approach is more aggressive than the simple mass or clonal propagation of elite individuals. In effect, the genetic stock is being "designed" or constructed with the goal being the improvement of traits important specifically for the SRWCP. This transformation of forest trees with foreign/recombinant DNA to improve the genetic characteristics of the plants must be seen as the ultimate goal of many tree improvement programs that are currently using tissue culture and molecular biology techniques. Estimates of the potential utility of tissue culture techniques in tree improvement programs have suggested that the gains from a breeding program may be realized from 8-18 years earlier than with conventional breeding techniques (Hasnain and Cheliak, 1986; McKeand and Weir, 1984). These gains would be the result of only the simple mass propagation of plants via tissue culture techniques (micropropagation and somatic embryogenesis). In addition, the ability to transfer foreign, recombinant, or modified genes into a forest tree could improve or add traits in ways not possible by any other means. The tissue culture propagation of these genetically transformed plants would dramatically reduce the time necessary to realize these gains. This rapid incorporation and utilization of improved or foreign long generation time/life cycle which hinders conventional breeding. This has prompted at least one investigator to state the obvious, that "the use of 'test tube' techniques for gene manipulation may have more use with woody crops than for herbaceous agronomic crops" (McCown, 1985).

The literature is replete with publications whose titles include the words "potential, prospects, or promise", which celebrate the merits of the application biotechnology to forestry (Durzan, 1985). Not until recently, however, has there been justification for such unbounded optimism. Any transformation/genetic engineering scheme relies on the successful completion of a series of general, although certainly non-trivial steps (Figure 5). The first is the identification and isolation of the gene(s) controlling the trait of interest. Second, the DNA controlling the trait must be transferred into the genome of the desired host plant. This transformation process often

A. Identification and characterization of trait

B. Isolation of gene (mRNA or DNA)

C. Clone gene (DNA)

D. Transfer gene (DNA) into host plant

E. Plant regeneration

F. Stable expression and transmission of new gene

G. Testing and assimilation into breeding program

Figure 5. Steps in plant genetic engineering.

requires the action of a biological shuttle or vector. The transformation schemes involving vectors described thus far require tissue culture manipulation of the host plant (Hinchee set al., 1988; Horsch, et al., 1985; Nester et al., 1984). Transformation methods not dependent upon biological vectors, such as electroporation, require the use of protoplasts as hosts (Shillito et al., 1985). Even DNA-coated microprojectile mediated transformation, used in the delivery of foreign DNA to cells in seeds, meristems and callus, requires the use of tissue culture methods to recover or propagate the resultant engineered plants (Christou et al., 1988; Klein et al., 1987; Klein et al., 1988; McCabe et al., 1988). This critical step/obstacle in the transformation process, therefore, involves the regeneration of the plant from cultured tissues, i.e., callus, suspension cultures, protoplasts, meristems or leaf disks. It is in this area that recent breakthroughs have occurred, which may render the application of genetic engineering/transformation of forest trees possible.

The year 1985 marked several significant breakthroughs for forest biotechnology. Hakman and Von Arnold (1985), using immature zygotic embryos as explants, produced somatic embryos of Norway spruce. Further progress with other explants and development of somatic embryos into plantlets was reported in later papers (Beewar et al., 1987; Hakman et al., 1985; Von Arnold and Hakman, 1986). Using megagametophyte tissue explants, somatic embryogenesis in calli of larch was also reported in 1985 (Nagmani and Bonga, 1985). These investigators also reported development of plantlets from somatic embryos. The methods described by these laboratories (most importantly the choice of immature embryos as explant sources) have been successfully applied to a wide variety of coniferous species (unpublished data, The Institute of Paper Science and Technology). In several respects, work with hardwood trees has a similar history. Reliable propagation through tissue culture has been reported for a number of angiosperm tree species, including aspen (Populus tremuloides; Wann and Einspahr, 1986), yellow-poplar (Liriodendron tulipifera; Merkle and Sommer,

1986), sweetgum (Liquidambar styraciflua L.; Sutter and Barker, 1985) and protoplasts of Populus hybrids (Russell, J.A. and McCown, B.H., 1986). Success with somatic embryogenesis should certainly reassure foresters that economically important forest trees can be regenerated in large numbers via tissue culture. These achievements then make feasible, if not practical, transformation and genetic engineering of forest trees. In fact, Agrobacterium-mediated transformation and regeneration of Populus hybrids has been reported several times. Both marker genes and the aroA gene, which imparts tolerance to the herbicide glyphosate, have been used (Parsons et al., 1986; Fillatti et al., 1987). Finally, optimism appears warranted.

Genetic engineering of forest trees is in its infancy. It would be presumptuous to describe, given the current state of knowledge, how biotechnology in general and genetic engineering in particular will impact genetic improvement of forest trees for SRWCP purposes. A traditional literature review confined to research or results reported in this area would be largely a fruitless effort. An alternative approach will be presented: Each of the arbitrarily defined steps in plant genetic engineering (Figure 5) will be discussed in terms of existing technologies, perceived difficulties, need for further work, and an opinion of which problems should be addressed first. Newly emerging technologies/methods that may be relevant to research and should be monitored will also be identified. This information should enable SRWCP to better define and identify future research strategies.

IDENTIFICATION OF TRAITS IMPORTANT TO FOREST TREES

In any plant improvement program, the foremost consideration relates to the choice of the trait to be manipulated or otherwise improved. This assumes, of course, that the target species has been chosen. The choice of trait(s) to be improved obviously relates to the end-use of the plant product. Hence, increased yield has been the focus of most crop improvement programs. Components affecting yield include growth rates, uniformity, nutrient efficiency, resistance to pests and diseases, environmental effects, etc. Generally speaking, breeding for improved (nutrient) quality has not been the case, with the exceptions being wheat (baking quality of flour) and the malting quality of barley. The quest for quantity over quality has been the driving force in most programs simply because of the economic rewards for quantity. Quantity of biomass has been the primary aim of the SRWCP. Factors to be optimized (investigated) have included growth rates, nutrient allocation (source-sink relationships), components of form such as branch angle and crown width, resistance to pests, stress and chemical herbicides. Since the use of the product may be greatly affected by both physical and chemical properties of the wood, this program will also consider wood quality characteristics. Specific gravity, fiber length, chemical composition, and nature of the chemical components may all affect ease of conversion to biofuels.

Those in tree improvement programs employing conventional breeding techniques are usually interested in the improvement of growth rate, form, wood quality (including fiber length and specific gravity), disease resistance and cold hardiness. With the exception of vertical disease resistance and possibly some aspect of form, these traits are

generally assumed to be under complex genetic control. The DNA for a trait controlled by a number of genes would be very difficult to isolate, using the techniques available today, and transfer into a host plant. Recently there have been suggestions, however, on how DNA (genes) involved in quantitative traits may be identified in a plant genome (Robertson, 1985). While further work may make genetic engineering possible, the state of the existing technology suggests that genetic transformation involving a complex trait, such as growth rate, may be very difficult if not impossible. A complex trait may be subdivided into individual components, though, that might be possible to modify. Light absorption by the leaf, branch angle, photosynthetic rates, photorespiration and assimilate partitioning are all characters which contribute to overall growth rate. These characters may be more simply inherited and amenable o transformation techniques, and if not, they themselves may be subdivided into components under simpler genetic control. It has been suggested, for example, that drought resistance in forest trees may be improved by modifying aspects of leaf morphology and/or stomatal response (Farnum et al., 1983). An example of this particular approach successfully applied to a crop plant was the identification of frost tolerant potato varieties. Frost-hardy Solanum species were found to have a leaf morphology characterized by two layers of palisade parenchyma cells, in contrast to non-hardy varieties which usually had only one distinct layer (Palta and Li, 1979). Nature of genetic control was not described, however, and utility may depend upon moving this trait into useful germplasm via traditional crossing.

Realizing the difficulties in improving a complex trait, molecular biologists have concentrated on characters controlled by single genes. Many of the first plant transformation studies imparted resistance to the antibiotic kanamycin.

Antibiotic resistance is understandably a model system, owing to ease of selecting transformed plants. Selection for herbicide resistance is also relatively easy to accomplish in vitro and in vivo. Basic work defining, on a biochemical and molecular level, resistance to the herbicides Roundup, Glean and Oust is underway. Resistance to glyphosate (Roundup), which disrupts aromatic amino acid biosynthesis, has been identified in the bacterium Salmonella. Resistance is imparted by a mutant aroA allele, with the gene consisting of a single open reading frame (Stalker et al., 1985). A plasmid vector containing this gene has been constructed, and under contract to the U.S. Forest Service, was used to transform a hybrid poplar at the commercial biotechnology company, Calgene (Fillatti et al. 1987b). The desirability of glyphosate tolerant hybrid poplars is yet to be proven, and may represent a forest tree genetic-engineering program attempted simply because "it could be done". Lack of industry and governmental interest in the plants produced by this work suggests that industry needs must be more carefully weighed in the future. This is not to detract from the utility of the program as a demonstration project, proving that foreign gene transformation of forest trees, hardwoods at least, is a reality. Obviously, the choice of trait is of great importance.

Other traits that are simply inherited may soon be the object of transformation studies. Resistance or tolerance to additional herbicides, phytotoxins, salt and heavy metals may be examples of these types of traits. The advantage of working with these traits is that selection may be easily carried out in tissue culture, which is invariably a component of transformation methods. Barton and Brill (1983) suggested that pest

resistance might be imparted to plants if the genes controlling the synthesis of toxic or noxious secondary products could be isolated and transferred into plants. Recent history has shown that approach to be useful, the gene encoding the insecticidal protein commonly known as "BT-toxin" (isolated from the bacterium Bacillus thuringiensis) and a protease inhibitor isolated from potato have both been transferred into host plants to confer resistance to insect pests.

Production of herbicide tolerant and pest resistant plants via genetic engineering are recent examples of successful application of molecular technology to plant improvement. Although these traits may give gains in productivity instead of feedstock quality, this work will be described to provide examples of how the techniques can be utilized in molecular manipulation of trees.

EXAMPLES OF SUCCESSFUL GENE TRANSFER

Herbicide tolerant plants may be obtained in a number of ways. Tolerant individual can be identified in breeding programs, variants can be identified in vitro (somaclonal variants), and tolerant transgenic plants can be produced (Chaleff and Ray, 1984). This discussion will be confined to the latter approach. To date, plants tolerant only to the commonly used herbicide glyphosate (Roundup) have been produced. Two strategies have been used. Workers at Monsanto Company have isolated the gene for the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) from an overproducing tissue culture cell line. This is the enzyme whose activity is inhibited by the herbicide Glyphosate-susceptible petunia cells and regenerated plants were made tolerant to the herbicide upon Agrobacterium mediated transformation with this gene driven by a 35s-cauliflower mosaic virus promoter (Shah et al., 1986). This work has been extended to several other plant species, most recently and notably soybean (Hinchee et al., 1988). Transgenic soybean plants were reported to exhibit significant tolerance to glyphosate and to transmit this trait to their progeny. This may become one of the first commercially useful applications of genetic engineering technology.

A second approach is taken by the research group at Calgene Incorporated. These researchers have isolated and cloned a gene from the bacterium Salmonella typhimurium, a mutant aroA gene, which confers resistance to glyphosate (Comai et al., 1985; Stalker, et al., 1985). This gene has been used to confer tolerance in tomato and hybrid Populus (Fillatti et al., 1987a; Fillatti et al, 1987b). To be most effective, however, the enzyme must be present in the plastids of the transformed plant. Since the protein product of a bacterial gene lacks the transit sequence required for import of the protein into the plastid, the degree of tolerance afforded by this gene was lower than expected. Recent reports suggest that this problem has been rectified.

It appears that glyphosate tolerant forest trees may soon be available on a scale required for field testing. This is not to discount the tolerant Populus (Fillatti et al., 1987b) which are undergoing field tests, but levels of tolerance currently available have not yet been shown to be useful in the field. As stated above, this is an area of forest

biotechnology that may hold promise if and when glyphosate tolerant plants become desirable.

The bacterium, Bacillus thuringiensis, synthesizes an intracellular protein which has insecticidal activity. This protein, the product of a single gene, has been isolated, characterized and cloned (Adang et al., 1985; Bulla et al., 1977). This gene has subsequently been introduced into recipient plants via Agrobacterium mediated transformation and has been shown to confer quite effective resistance to lepidopteran pests (Barton et al., 1987). Two recent reports have shown the foreign gene to impart insect resistance in regenerated tobacco as well as tomato plants (Fischhoff et al., 1987; Vaeck et al., 1987) If a target species of the SRWCP is subject to predation by an appropriate insect pest (lepidopteran), it might then be feasible to consider the incorporation of this gene into a forest tree species. At present, however, there appears to be a question as to whether or not this gene would be economically valuable to the SRWCP.

A clever approach to the protection of transgenic plants against damage by viral pathogens has been used by Roger Beachy's laboratory at Washington University inSt. Louis. Pathologists had known that a minor infection or an infection with a mildly pathogenic virus would protect the plant from subsequent infection of a more virulent strain. It was found that this "viral cross protection" could be conferred in plants not only by a prior infection with a mildly virulent strain, but also if specific viral coat proteins were present in the cells of the plant. Beachy's group, and now others, cloned the gene encoding a viral coat protein and introduced this foreign gene into various plants via Agrobacterium-mediated transformation. Both tobacco and tomato plants were transformed with a gene encoding a tobacco mosaic virus (TMV) coat protein (Abel, et al., 1986; Nelson, et al., 1986). The transgenic plants were found to have a very high level of resistance to controlled viral infections and were able to transmit this character to their progeny in a Mendelian fashion. The transgenic tomato plants were found to be resistant to not only TMV, but tomato mosaic virus (ToMV) as well. Viral cross protection resulting from the expression of foreign genes encoding coat proteins has recently been used to confer resistance to potato virus X and cucumber mosaic virus (Cuozzo et al., 1988; Hemenway et al., 1988). A similar strategy to protect against viral infection involves the expression of viral satellite RNA in the transgenic plants (Baulcombe et al., 1986). The presence of viral satellite RNA has been shown to modulate the severity of symptoms caused by viral pathogens (Simon, 1988). The lower degree of protection and risks involved in this approach render the "coat protein" approach used by Beachy's group the preferable method at this time.

Synthesis of certain proteins is induced upon infection by pathogens or physical stress/damage in crop plants. Several proteins isolated from bean, maize, tobacco and potato have been found to have chitinase or gluconase activity (Hedrick, et al., 1988; Kauffmann et al., 1987; Kombrink et al., 1988; Nasser et al., 1988). Protease inhibitors, thought to play a role in the defense against insects, have also been isolated, cloned, and partially characterized (Joudrier et al., 1987). Use of genes encoding protease inhibitors to protect plants is being considered. Trypsin inhibitor, a protease inhibitor from cowpea, is known to protect seeds from insect predation.

Hilder et al. (1987) have cloned and transferred this gene into tobacco plants. The regenerated tobacco plants were found to not only express detectable levels of the foreign protein, but were also protected from defoliation by tobacco hornworms introduced under controlled conditions. This approach may possibly become useful in future forest tree improvement schemes. Agin, this presumes that the total yield of biomass will be positively affected by the incorporation of insect resistant individuals into the energy plantation.

SOME NEW APPROACHES

A relatively new approach to study and elucidate function of genes, or to disrupt the synthesis of a specific gene product may prove a powerful tool in plant improvement programs. The method involves construction of a recombinant DNA molecule in which a promoter is placed on the 5'-end of a non-coding strand of DNA. After transcription, the resulting "antisense RNA", which is complementary to normal or "sense" RNA, is able to anneal or hybridize with the target mRNA. Translation is thereby inhibited or blocked, resulting in low levels of gene expression. Production of antisense RNA to affect translation of a gene is a normal aspect of the regulation of gene expression by prokaryotes (Green et al., 1986; Mizuno et al., 1984). Initially, antisense RAN was synthesized in vitro and injected into cells in order to demonstrate effects upon translation. Later, antisense constructs were used to transform cell lines to suppress expression of both foreign and endogenous genes in recipient cells. The reader is referred to the excellent recent review on the utility of antisense genes and for a more complete historical treatment of this quickly advancing field (Krol et al., 1988a). In studies on the expression of thymidine kinase (TK) in animal cells, antisense constructs as short as 52 bases have been shown to effectively reduce expression of TK (Izant and Weintraub, 1985). The antisense transcript was homologous to a region in untranslated TK mRNA, however, suggesting how such a short antisense sequence could so dramatically disrupt translation. This finding is notable, though, as it illustrates how simple an effective antisense sequence can be. A short antisense sequence would be much simpler to construct than a sequence encoding the entire mRNA. Short sequences have also been shown to maintain specificity, as suppression of TK expression was species specific (Izant and Weintraub, 1985).

Effect of antisense RNA on gene expression in plants has been the subject of a growing number of reports (see table 4 in Krol et al., 1988a). First experiments employed antisense sequences homologous to marker genes for chloramphenicol acetyltransferase (CAT) and nopaline synthase (Delauney et al., 1988; Ecker and Davis, 1986; Rothstein et al., 1987; Sandler et al., 1988). The antisense RAN clearly suppressed formation of these foreign gene products. Recent work has been on the effect of antisense RAN on the expression of endogenous genes. Chalcone synthase (CHS) is an enzyme involved in the formation of colored compounds in the flowers of various species. Both petunia and tobacco plants have been transformed via Agrobacterium carrying an antisense CHS construct (Krol et al., 1988b). Flower coloration was dramatically affected in regenerated transformed plants. Notable,

however, was the finding that the efficiency of the antisense RNA varied between and even within tissues in individual transformed plants. Variation in the coloration of a given flower was observed, suggesting differences in action of the antisense RNA between sectors. Tomato plants have also been stably transformed with an antisense gene. Antisense polygalacturonidase, an enzyme involved in the ripening process of tomatoes, effectively inhibited the expression of the enzyme (Smith et al., 1988). In their review, Krol et al. (1988a) also cite unpublished data on the inhibition of the synthesis of the small subunit of ribulose-1,5-bisphosphate carboxylase. This has provided a new technique to study the relationship between synthesis of the plastid encoded large subunit and the nuclear encoded small subunit.

Several research groups have attempted to confer protection to viral plant pathogens via antisense RAN technology. Antisense constructs of coat proteins of both cucumber mosaic virus and potato virus X have been integrated into recipient plants (Cuozzo et al., 1988; Hemenway, et al., 1988; Rezaian et al., 1988). The results have been disappointing, however, as the degree of protection afforded by the antisense RNA has been low. It appears that the viral cross protection schemes described by Beachy's group are much more effective.

For the near future, it is difficult to see where this technology could be used in the production of transgenic tree species with properties improved for use in the SRWCP. Work on the basic understanding of the growth and physiology of tree growth, stress responses, and the better characterization of wood and its primary constituents (cellulose, hemicellulose and lignin) must be carried out as a prelude to the application of this technology. Antisense RNA can be used as a tool to elucidate the function of a particular gene on wood quality, however. Cyclic-AMP has long been known to be involved in the aggregation of Dictyostelium discoideum cells in the formation of a multicellular reproductive body. Proof of this action of cAMP was provided by an experiment in which Dictyostelium cells were transformed with an antisense construct homologous to the cell surface cAMP receptor (Klein et al., 1988). The transformed cells did not produce the receptor, and more importantly, failed to aggregate upon stimulation by cAMP. This experiment illustrates how powerful this technique can be in determining the role or function of a gene. The use of antisense RNA may be somewhat analogous to the use of mutants to dissect the role of a gene. Obviously the use of mutants to determine the role of genes on forest tree growth and w ,od properties is limited by lack of mutants.

Perhaps antisense RNA technology could be applied to the study of specific genes in forest trees. The effects of the full or partial suppression of lignin biosynthesis, for example, in transformed tree cells or regenerated plants might be of considerable interest. Although the ability to affect lignin biosynthesis with antisense RNA technology is speculative at the moment, it is possible to suppress the expression of genes for specific enzymes. As discussed earlier, PAL is an enzyme intimately involved in the supply of substrates for the formation of lignin. It might be very informative to disrupt, or at least suppress, PAL expression in a woody plant to demonstrate the role or importance of this enzyme. Certainly this area deserves continued attention and perhaps application to tree species.

TRAITS RELATED TO WOOD QUALITY AND COMPOSITION

A previous section on the biochemical nature and regulation of several traits of interest to the SRWCP painted a less than satisfying picture. Even though the primary constituents of wood (cellulose and lignin) comprise a major proportion of the biomass on the surface of the earth, relatively little is known about the synthesis and biochemical control/regulation of these compounds. Although much is known about the formation of the precursors of cellulose and the ultrastructural details of microfibril formation (reviewed in Delmer, 1987), the enzyme complexes responsible for the biosynthesis have yet to be purified/characterized, and in vitro synthesis of cellulose has still not been attained. It is most probably that the rate of cellulose biosynthesis, the nature of the cellulose, the areas of cellulose deposition etc. are traits controlled by a (large) number of genes. The same is true for most other traits of interest. Wood density, ash content, bark quality and quantity, and extractives are poorly defined and characterized on a genetic level. As illustrated in the discussions about conventional improvement, heritabilities of most such traits have not only been crudely estimated, but are generally low. The polygenic (quantitative) control, lack of understanding or characterization, and nature of inheritance of these traits does not render them amenable to genetic manipulation on a molecular level at the present time. Before manipulation of a trait can be executed at a molecular level, the biosynthetic pathways and regulatory aspects must be understood. Thus, work in this area needs to be focused not on the molecular manipulation of the trait, but rather on the basic characterization of these traits. Without a basic understanding of the physiological, biochemical and molecular (genetic) levels, it is premature to consider manipulation of these traits using genetic engineering techniques. Simpler studies of perturbation and expression of enzymes or proteins impacting the trait can be conducted now, however (i.e. see section on antisense-RNA).

Lignin Formation

Some headway is being made on understanding of the nature and regulation of lignin biosynthesis. Some of the research that may impact the molecular genetic manipulation of lignin has not addressed lignin per se, however. Phenolic compounds are often involved in the response of a plant to injury or infection by a pathogen. Much work has understandably, then, concentrated on the phenolic compounds and the enzymatic control of their synthesis. Phenylalanine ammonia lyase (PAL) has receive significant attention as it is one of the rate-limiting enzymes controlling the flux of precursors into phenolic acid biosynthetic pathways. PAL has been isolated and cloned from several plant species, as have other enzymes involved in the metabolism of phenylpropanoids (recently reviewed in Mol et al., 1988). Chalcone synthase (CHS), an enzyme involved in the formation of flavonoids, has also been characterized and cloned (Koes et al., 1987; Mol et al., 1988; Sommer and Saedler, 1986) and has been utilized in studies on the utility of antisense RNA as mentioned above (Krol et al., 1988b). Even though the goal of of these studies was to understand the regulation and induction of these compounds as part of a stress response, much of the work may be applicable to

those interested in the formation of lignin. As yet, we are uncertain what effects modifying the expression of PAL, or other enzymes involved in the formation and modification of phenolics such as o-methyltransferases, will ultimately have on the formation and nature of lignin. Using this approach in an experimental sense, however, may result in a better understanding of the biosynthesis of lignin and facilitate more practical work.

Growth Regulators

It is generally assumed that most, if not all, of the traits of interest to the SRWCP are under complex genetic control (bearing in mind the aforementioned exceptions such as vertical disease resistance). A characteristic such as lignin biosynthesis is probably subject to the control of a myriad of genetic as well as environmental factors. This does not preclude the fact, however, that a simple modification of one physiological or biochemical parameter may have significant effects on it. A photograph on a cover of Science magazine dramatized this point. In a 1983 report, Palmiter et al. (1983) demonstrated the expression of a foreign gene in mice, the gene consisted of a mouse metallothionein promoter fused to a human growth hormone (GH) coding sequence. The transgenic mice exhibited increased levels of GH compared to control mice. Most dramatic, however, was a photograph of a transgenic mouse beside a control mouse. The transgenic mouse was more than twice the size of his untreated sibling. This work illustrates that something as complex as body size, or lignin synthesis, might be accessible to manipulation. While the comparison of the manipulation of growth rate/body size in animals and plants may not be may not be legitimate due to the absence of a plant "growth hormone" per se, there is evidence that levels of a single plant growth regulator can significantly influence growth rate and other characteristics.

A cross of two inbred maize lines can result in a hybrid with increased yield, growth rate, etc. Although the the precise nature of this heterosis (hybrid vigor) is not known, a recent report has suggested that the level of plant growth regulators, gibberellins (GAs), may be one component of this increased performance (Rood et al., 1988). Levels of endogenous gibberellins were found to be significantly higher in the hybrids, compared to the inbred lines. Interestingly, application of exogenous GA to maize plants preferentially increased growth in the inbreds. This data suggests, then, that increased levels of GA are at least partially responsible for the vigor of the hybrids.

Similar results have been obtained in studies of Populus species. A positive correlation was found between the concentration of GAs and hybrid vigor for both height growth and dry weight (Bate et al., 1988). In addition to effects on growth rate, growth regulators have also been shown to control wood quality characteristics. Indole-acetic acid (IAA) has been shown to regulate the nature of the hemicellulosic polysaccharides in growing pine tissues (Lorences and Zarra, 1987). IAA led to decreases in the molecular weights of cell wall xyloglucans in this study.

These studies suggest that levels of endogenous growth regulators probably control many of the traits of interest to SRWCP. It is also becoming apparent that levels of growth regulators might be subject to manipulation via molecular genetics. Smigocki and Owens (1988), for example, have shown that cytokinin production was elevated in plants transformed with a foreign isopentyltransferase gene. This is among the first attempts to manipulate levels of growth regulators in transgenic plants, and certainly deserves continued research. Manipulation of endogenous growth regulators may find use in production of stock more suitable for SRWCP.

OTHER CONSIDERATIONS

An important issue in genetic transformation of plants is not only choice, isolation, cloning and transfer of the foreign gene into the host plant, but also regulation of that gene in the foreign environment. Genes are now known to consist of more than a simple DNA sequence encoding the formation of a protein (see discussions in texts by Freifelder, 1983; Alberts et al., 1983). Sequences both upstream and downstream of the translated coding sequence or open reading frame (ORF), although not transcribed, are essential for gene expression. The upstream elements, promoters, dictate both level and timing of gene transcription. Promoters may stimulate continuous transcription of a gene, which is constitutively expressed, or be regulated by developmental, physiological or environmental factors. Studies using marker genes in experimental transformations have generally used genes fused to constitutively expressed promoters. A number of promoters are commonly used, several being derived from the cauliflower mosaic virus (e.g. 19s, 35s promoters), while others have been isolated from the T-DNA of Agrobacterium (nos promoter). While continuous expression of a foreign gene might be desired in transgenic plants carrying a gene modifying lignin quality, it may also be advantageous to regulate or control the expression of the foreign gene. This could be accomplished by fusing the foreign gene with a promoter under the control of a known stimulus. Several promoters of this type have been isolated and characterized. Promoters under the control of plant growth regulators, heat shock, stress and light have been shown to regulate expression of a foreign gene (Kuhlemeier, et al., 1987; Marcotte et al., 1988). The sequence down-stream, or 3', from the ORF are responsible for the polyadenylation of the RNA transcript, which is important for stability and transport of mRNA. Several of these sequences have been used in gene fusions and have been shown to allow the expression of foreign genes in transgenic plants. To summarize this point, both constitutively expressed and regulated gene promoters, as well as polyadenylation signal sequences, have been isolated, characterized and used in plant transformations. These regulatory sequences appear to result in reliable expression of foreign genes in plants, and will probably be sufficient for the expression of genes in forest trees; Fillatti et al. (1987b) have already demonstrated the expression of foreign genes in Populus hybrids, for example, In short, this aspect of the technology is mature enough to be immediately applied to the transformation of forest trees for SRWCP.Related to the expression of foreign genes in eukaryotic cells/organisms, another important aspect that must be considered is the cellular destination or location of the gene product. A large number of gene products are soluble proteins or enzymes found in the cytoplasm. Many proteins, however, serve as integral parts of chloroplasts and

mitochondria, even though they are encoded in the nuclear genome. Proteins that are destined to be transported across the membranes of these organelles are "tagged" or identified by transit or signal peptides. These short peptides, formed on the end of the proteins as part of the normal translation of the mRNA for the proteins, ensure that the proteins will "dock" onto the organelle and are then cleaved off as the protein enters the organelle. Owing to the importance, abundance and ease of manipulation of the protein, considerable study of the transport of the small subunit of ribulose-1,5-bisphosphate carboxylase into plastids has been undertaken (Chua and Schmidt, 1978; Highfield and Ellis, 1978). The DNA sequence for the transit peptide of the small subunit has been fused to foreign marker genes, such as the bacterial nptII gene, and the foreign proteins have been imported into the chloroplasts (Cashmore et al., 1985; Van den Broeck, et al., 1985). Sequences encoding for transit peptides of other nuclear-encoded plastid proteins have been characterized, and show significant similarities (Schmidt and Mishkind, 1986). It appears, then, that fusing these sequences to the foreign genes that must be imported into plastids will successfully facilitate their intracellular transport. Similar work has been done with respect to the import of proteins into mitochondria. Dihydrofolate reductase (DHFR), a cytoplasmic protein, isolated from mouse has been transported into yeast mitochondria when fused to the appropriate leader sequence (Hurt et al., 1985). All of the above described findings suggest that foreign proteins can be targeted to the chloroplast or mitochondrion almost at will. These results may become significant to the goals of the SRWCP if the genetically modified or foreign protein must be localized in one of these organelles.

In addition to the required localization of specific nuclear-encoded proteins in plastids and mitochondria, other proteins must undergo post-translational processing or modifications before they can function or be utilized. Many proteins, for example, are glycosylated in their mature forms. Many of the modifications of this type occur in the endoplasmic reticulum and Golgi bodies (Farquhar and Palade, 1981; Rothman, 1985). It is becoming increasingly apparent, then, that before a foreign gene is transferred into a tree, the protein encoded by the gene must be well characterized. Steps will have to be taken to account not only for intracellular location of the protein, but also for any post-translational modifications.

ISOLATION OF GENES (mRNA or DNA)

There are several general strategies employed to isolate a gene (DNA) so that it may be cloned in a bacterial vector. The first approach involves purifying and sequencing the protein product of the gene of interest. Using the amino acid sequence data obtained from the characterization of the protein, a collection of oligonucleotide probes homologous with the gene is constructed, which are used to screen a gene library.

A second method involves purification of relatively large amounts of the target protein and isolation of antibodies to this protein. These antibodies are then used to screen the library in an expression vector. Genes in the library are all being expressed in individual colonies/plaques of bacteria infected with a bacteriophage. The bacterium

transcribes the foreign gene, the resulting RNA is then translated by the bacterium, and the antibody used to screen the library is used to identify the colony/plaque producing the target protein. This desired colony, comprised of bacterium carrying the gene of interest, is then isolated and used in isolation of mass quantities of the gene. This technique suffers the limitation that post-translationally modified proteins will not be processed by the bacterial vector. This may complicate antibody screening.

A third approach, perhaps the simplest, involves the use of a heterologous probe to screen the genomic library. This involves the use of a pre-exiting DNA probe, obtained from another species, homologous to the gene of interest. This approach could be used to clone a well characterized, highly conserved gene for rubisco, for example. If the goal was to clone a willow gene, for instance, the easiest approach would be to acquire the cloned gene from a laboratory that had cloned it from another plant. This sequence would probably have enough homology with the gene in willow so that it could be used as a probe with which to identify the sequence in a willow library.

These techniques could be explained in greater detail, but such a discussion is beyond the scope of this review and would simply reiterate what is available in molecular biology lab manuals (i.e. Maniatis et al., 1982; Mantell et al., 1985; Old and Primrose, 1985). Since these methods are well understood for bacterial, animal and crop plant species, perhaps the construction of cDNA and genomic libraries of forest trees should commence. This will insure that the technology will be ready when the need arises. There is some evidence that library construction with forest trees will require modification/optimization of standard techniques. Problems with large genome sizes, extractives, and difficulties in isolation of high quality DNA (i.e. sweetgum, F.J. Lang, Institute of Paper Science and Technology, personal communication) may complicate library construction and screening/cloning.

Cloning Genes (DNA)

Of all the steps defined for genetic transformation, mass production (or cloning) of a particular DNA sequence is the most routine. It is by no estimation a trivial step or process, but the cloning of a gene is an exercise that can be done in an advanced undergraduate biology/biochemistry course. Sequences are usually cloned in either bacterial plasmid vectors or in bacteriophage vectors. An abundance of cloning vectors, adapted to many cloning strategies, are available commercially from a number of companies such as Pharmacia, Bethesda Research Laboratories, Promega and Stratagene. Techniques for preparation of cloning vectors, ligation of the target DNA into the vector, the transformation of the host E. coli, isolation of mass quantities of the gene from the bacteria and further manipulation of the DNA sequences are described in many of the standard molecular biology "methods notebooks" available. The reader is referred to the handbooks authored by Maniatis et al. (1982), Davis et al. (1986), and Berger and Kimmel (1987). Especially germane to those working with plant genes are the plant molecular biology manuals of Malmberg et al. (1985) and Gelvin and Schilperoort (1988). This aspect of the biotechnology deserves little research attention of funding.

Transfer of Foreign Genes into Plant Cells

Two general systems are currently under consideration for the movement of foreign or recombinant genes into plant cells, biological vectors and electroporation. Although a number of micro-organisms which infect plant cells, such as the Caulimoviruses and Gemini viruses, may potentially be used to transfer DNA into trees, most work has centered on the Ti-plasmid of Agrobacter tumefaciens. The use of Agrobacterium as a vector has been the subject of a number of recent reviews (Klee et al., 1985; Nester et al., 1984) in which the biology and molecular biology of the bacterium and the plasmids it harbors have been described. Briefly, upon infecting the plant a plasmid is transferred from the invading bacterium into the plant cell. Portions of this plasmid are then incorporated into the nuclear genome of the plant. This was the system used to transfer the aroA gene into popular trees (Fillatti et al., 1987). A potential problem in the use of Ti-plasmids, however, relates to the host range of the Agrobacterium. Certain strains of the bacterium have very limited host ranges, and may be unable to infect the desired host plant. The host range of the bacteria is being studied and has been found to be under genetic control (Yanofsky, et al., 1985). Fortunately, most forest trees are susceptible to Agrobacterium (DeCleene and DeLey, 1976). Infection of tissue of tissue cultures or excised sections of plants is also possible, and infection of leaf disks to transform plants has become routine (Horsch et al., 1985).

Another method used to genetically transform plant cells involves the incorporation of naked DNA into protoplasts. In this method, apparently modified for use with plant cells in the labs of Potrykus and Walbot simultaneously, a brief electrical pulse is applied to a mixture of protoplasts and DNA solution (Fromm et al., 1985; Fromm et al., 1986; Shillito et al., 1985). Although the exact mechanism of the technique is unknown, it is postulated that the electrical pulse opens "pores" in the plasmalemma, through which the foreign DNA migrates. Once inside the cell, the DNA may then be incorporated into the nuclear genome of the plant cell. Due to the proposed mechanism of the technique, it has been named electroporation. Although efficiency of successful incorporation of foreign DNA may be low, this problem is counteracted by the large populations of protoplasts that may be used. Herein lies the current limitation of electroporation of trees, however. Protoplasts of the tree must be available, and it must be possible to regenerate intact plants from these protoplasts if the gains from this process are to be realized. To date, regeneration of protoplasts remains an art, although notable success with certain tree species (Populus hybrids) has been achieved (Russell and McCown, 1986).

A relatively new technique to deliver foreign genes into plant cells involves a microprojectile, or bullet-gun, system. Klein et al. (1987) pioneered a technique in which tungsten particles were coated with the DNA to be transferred, the particles were then accelerated by a small gunpowder charge striking and entering the target cells. In the first trials marker genes were delivered into intact epidermal cells of onions. Procedures using microprojectile mediated transformation continue to be optimized. This is exemplified by work on the transformation of intact rice, wheat and soybean cells

with GUS and CAT marker genes (McCabe et al., 1988; Wang et al., 1988). The system has evolved so that the particles, gold instead of tungsten, are accelerated in a more controllable fashion by an electric discharge gun (Christou et al., 1988). In addition to these species, this transformation technique has also recently been used to transform maize (Klein et al., 1988).

Other techniques used to effect foreign gene transfer into plant cells have been discussed in a more theoretical or speculative flavor, such as chromosome mediated transfer (Klobutcher and Ruddle, 1981), but these techniques remain unproven. At present, either electroporation, microprojectile or Agrobacterium mediated transfer appear to be the methods most likely to succeed in forest tree genetic engineering schemes.

Regeneration of Forest Trees from Tissue Cultures

The regeneration of forest trees from cuttings, tissues or organs has been possible for a number of years and has been the subject of several reviews (Durzan, 1985; Farnum et al, 1983). The review by Karnosky (1981) contains tabular lists of both angiosperm and gymnosperm tree species that have been regenerated in vitro. It must be noted, however, that the lists consist of species that could undergo organogenesis, that is, the formation of a shoot and the subsequent rooting of the shoot to form a complete plant. Organogenesis is often done on the original explant, i. e., excised cotyledon, embryo or leaf segment. In light of the report by Horsch et al. (1985) in which leaf disks were successfully transformed using the Tiplasmid system, organogenesis may prove to be sufficient to produce limited numbers of transformed forest trees. An alternative approach involving somatic embryogenesis of the cultured trees may hold more promise for the mass production of transformed/genetically engineered plants. Most forest trees, especially the conifers, have proven to be recalcitrant in culture and at the time that Karnosky's review was written somatic embryogenesis had not yet been achieved for any conifer used in the forest industry. The recent advances in somatic embryogenesis of forest trees were described earlier in this report, and seemingly influence one to conclude that the time is now ripe for the genetic transformation of trees to proceed with increased vigor. however, it must be recognized that somatic embryogenesis has been achieved only using embryos, many times immature, and seedline tissues. These plants are of unknown genetic composition, and their mass propagation should be viewed with caution. It is clear, then, that much additional work is needed on the initiation of cultures from truly mature, genetically elite sources.

Stable Expression and Transmission of New Genes

The inheritance of foreign genes in regenerated, transformed plants has been examined in a number of plant species/transformation systems. Many reports on the successful transmission and segregation of foreign genes provide a sense of optimism as Mendelian inheritance was observed in the progeny of transgenic plants produced via direct gene transfer methods (Morota and Uchimiya, 1988; Uchimiya et al., 1986) as well

as with Agrobacterium-mediated gene transfer (Budar et al., 1986; Feldmann and Marks, 1987; Horsch et al., 1984; Horsch et al, 1985). In at least one recent report, however, a significant number of transgenic plants did show non-Medelian transmission of the foreign gene (Delores and Gardner, 1988a). A number of the plants that exhibited decreased transmission of the gene were found to contain the foreign DNA, but levels of expression of the gene were low enough to complicate the scoring of the plant as transgenic. Other plants, however, simply showed non-Mendelian transmission of both the trait (gene expression) as well as the physical gene (DNA).

A factor influencing the stability and subsequent transmission of the foreign gene is the site of insertion of the foreign DNA into the genome of the host plant. In addition to this, alterations or modifications of the DNA obviously influence the expression and perhaps heritability of the gene. Other potential problems in the transformation of trees relate to the uncontrolled nature of the transformation event itself. Control of the position of insertion into the genome and the copy number may be difficult and unpredictable (Flavell, 1982). The location of the insertion of genes into host-plant DNA is uncontrolled and presently appears to be random (Chyi et al., 1986; Feldmann and Marks, 1987; Jones et al., 1987; Jorgensen et al., 1987; Spielmann and Simpson, 1986; Wallroth et al., 1986). One or multiple copies of the T-DNA may become incorporated into the genome of the host plant (Zambryski, et al., 1980). In Agrobacterium-mediated transformed petunia plants, T-DNA border sequences were often rearranged or deleted, without having severe detrimental effects upon expression or heritability (Delores and Gardner, 1988b). That study did report, however, that multiple insertion of T-DNA negatively affected transmission of the foreign genes. Thus far, the random insertion of foreign genes into the genomes of host plants has not been a severe limitation on the technology. Techniques allowing the targeting of genes or DNA to specific regions of the recipient's genome might someday be possible, through a controlled homologous-recombination approach for instance. With regard to the production of transgenic plants this approach remains in the realm of speculation, however. In species having unusually large genomes, such as many coniferous trees (Price et al., 1974), there appears to be an inordinately large amount of heterochromatic, repetitive or inactive DNA (Kriebel, 1985; Miksche and Hotta, 1973). The effects of foreign genes integrating into these areas of the genome on the production of transgenic plants (conifers) are not clear at present.

The above discussion on heritability of foreign genes was based upon the assumption that transmission of the foreign DNA from engineered plants to their progeny was desirable. Given the regulatory and political climate, this may not be the case, and the opposite may in fact by true. It may become important to limit the propagation and/or dissemination of foreign genes into natural populations. Perhaps the easiest way to accomplish this is by preventing dispersal of pollen and seeds. Those working with crop plants would probably incorporate a system of male sterility, including the use of maintainer lines and restorer genes. Since male steriles are not available to forest tree breeders (G. Wyckoff, IPC staff, personal communication), an alternative approach may have to be devised. The pollen/seeds could be physically contained, although this would be difficult with Populus species given the nature of seed dispersal. Disruption of pollen/seed development would result in containment. In dioecous species,

male steriles or plants incapable of seed formation would be required. In monoecious species, completely sterile individuals would be necessary. Either would require sterile mutants. As (male) sterile individuals have been found in most crops, they probably exist in forest trees as well. The problem lies in finding and identifying those individuals in wild or cultivated populations. An alternate approach would be to "engineer" sterility into the species. Since sterile mutants, somaclonal or induced would be difficult if not impossible to identify in regenerated plants, a more specific approach would have to be taken. If a gene, or protein, was identified as being essential to the meiotic process or flower formation, then this gene would be the target of manipulation. Antisense RNA, discussed earlier, might be used to disrupt expression of that gene, in effect resulting in a sterile individual. This is but one of several speculative approaches that could be tried.

Testing and Assimilation into Breeding Program

Tissue culture techniques are not without problems. Mortality of loblolly pine seedlings derived from organogenesis has been greater than that from seeds (Leach, 1979). Protocols insuring successful establishment of tissue culture plants in the greenhouse and test field plantings continue to be improved (Selvapandiyan et al., 1988). Haploid explants have produced a large proportion of diploid and mixiploid organs/structures in Norway spruce organo genesis from female gametophyte tissue (Simola and Honkanen, 1983). Limited reports concerning diploid explants of pine, in contrast, have demonstrated that nuclear DNA appears to be genetically stable, at least as judged by karyotype analysis (Renfroe and Berlyn, 1984). Tissue culture induced variation in regenerated plants, termed somaclonal variation by Larkin and Scowcroft (1982), was pronounced one advantage in application of tissue culture to crop improvement (Larkin et al., 1984; Scowcroft and Larkin, 1983). A number of subsequent reports have shown that variation induced in tissue culture is no greater than that observed in progeny of sexual crosses (Pfeiffer and Bingham, 1984). The value of the induced-variation aside, however, one has to consider possible detrimental effects of somaclonal variation. It is entirely possible that deleterious variants would be introduced into populations of regenerated plants. Also, one can assume that in any genetic transformation program, the foreign genes would be inserted into selected, elite genetic backgrounds. The induction of unwanted variation in the regenerated plants resulting in non-uniform (unproven) populations is undesirable. Tissue culture induced variation may pose greater problems for trees, which may show deleterious traits only after years of growth. Obviously somaclonal variation must be controlled or eliminated in tree improvement based upon genetic engineering. Unfortunately, causes and means to control somaclonal variation are poorly understood (Lee and Phillips, 1988). This issue must be better understood before genetic transformation of forest trees becomes routine.

Recommendations

During the preparation of the preceding sections, it became increasingly obvious where additional research work is necessary, if not essential, to make molecular

manipulation of traits in forest trees a reality. In a number of areas, techniques developed with animals or plant groups not including woody plants could be immediately applied to further SRWCP goals. These will be identified in the "Near Term Goals" section below. In the discussions on the identification and characterization of traits, a large amount of basic background work was indicated as needed before such traits can be modified. This type of work, longer term in nature, will be considered in the section on "Long Term Goals."

Near Term Goals. Several lines of research are "mature" enough to be applied.

1. The potential utility of antisense RNA technology is great, both in the modification of target genes and also in the elucidation of the function of a specific gene or gene product. Antisense constructs of the enzymes, PAL and CHS, both of which are involved in the production of substrates for lignin formation, could be used to determine the role of these enzymes. Inhibition of PAL, for instance, might disrupt synthesis of lignin precursors to the extent that lignin synthesis is blocked. Aside from the potential reduction of lignin in the biomass, a goal of the SRWCP program, this would also allow the study of plants that lack this important cell wall constituent. Since antisense RNA to both of the enzymes could be acquired relatively easily, this work could proceed as soon as possible.

2. Genes for the synthesis of growth regulators are becoming available, isolated mostly from Agrobacterium, which are being used in foreign gene transformations. Genes leading to the biosynthesis of both auxins and cytokinins, both isolated from A. tumefaciens, have been transferred into model plant species (Medford and Klee, 1988). These genes could just as well be transferred into woody plants, not only to determine if the gene elevated the level of growth regulators in transgenic plants, but also to test effects of elevated levels on quality or growth. Again, the foreign genes exist, and could easily be transferred into woody plants. This too should be the focus of future research.

3. An approach taken by several research groups, including an interdisciplinary group at Michigan Technological University, is an attempt to modify the biochemical nature or composition of lignin. These groups are attempting to modify the syringyl/guaiacyl subunit ratios in the lignin, presumably to affect the pulping quality of the wood. As discussed in the biochemistry section of this review, changing these ratios might also enable easier conversion to biofuels. This area of research is being pursued, although with other goals in mind, and should receive continued attention.

4. A research topic not previously discussed in this review is the utility of molecular-benetic markers to both identify desired individuals in populations and to isolate the gene(s) responsible for a specific trait. Restriction fragment length polymorphisms (RFLPs) are finding phenomenal use in the field of predictive genetic disease research in humans (White and Lalouel, 1988). The utility of RFLPs as tools in crop improvement programs has been discussed by Helentjaris et al. (1985), with the first applications including the association of specific RFLPs with important traits in tomato. Osborn et al. (1987) identified RFLPs linked to soluble solids content in tomato, which will greatly accelerate selection and breeding of this trait. It has been suggested that

RFLPs might also aid in the genetic characterization of quantitative traits (Edwards et al., 1987). Clearly, RFLPs could be used not only to speed selection of traits important to the SRWCP, but might also aid in the characterization and isolation of genes so that they may be manipulated via molecular-genetic means. There is no reason why the search for RFLPs in woody plants cannot be initiated now.

Long Term Goals. There is a glaring lack of basic information on most of the traits important to the SRWCP. Below are listed several areas of research that need immediate attention. That is not to say, however, that the required information will be generated in a short amount of time. These endeavors will require long-term commitments.

1. Regulation of biosynthesis and deposition of the main constituents of wood (cellulose, hemicellulose and lignin) must be better defined before these traits can be manipulated on a molecular level. This also holds true for other traits such as specific gravity, water and ash content. A better physiological, biochemical, as well as genetic understanding of these traits is needed. Enzymes responsible for their regulation need to be identified, characterized and cloned. Only then can manipulation of these traits proceed in a rational sense, at least on an experimental basis.

2. Nutrient allocation/photoassimilate partitioning is an area of crop plant physiology that receives considerable attention, in both industrial and government research programs. The nature of the allocation of carbon to stem or leaf (or to be more relevant to the SRWCP...bole or branch) has a significant impact on the quantity and quality of the biomass. Source-sink relationships and the regulation of the partitioning of carbon in growing plants has been the subject of much research (see symposium review of Preiss and Heath, 1985), but most of this work has been done with crop plants. Clearly, more work of this type needs to be done with woody plants, especially on how these factors affect wood quality. A related, although more nebulous concern is the issue of photosynthetic efficiency. Again, studies on photosynthetic efficiency and biomass accumulation are being done with crop plants (Bugbee and Salsbury, 1988), but the characterization and manipulation of the metabolic efficiency in forest trees is lacking. Characterization and possible control of photosynthesis is obviously a long range research topic, but may merit attention.

3. Somaclonal variation is viewed by some as an advantage of the plant tissue culture process, but unwanted variation must be eliminated and controlled in the production of transgenic plants. A large amount of work will be needed to better identify, let alone control, this variation. Since study of somaclonal variation in forest trees may be complicated by their long life cycle, long term studies comparing explant sources and tissue culture regenerants should begin now. This approach is being used at the Institute of Paper Science and Technology to compare growth and other characteristics of a conifer seeding with plants regenerated from cultures initiated from that specific seedling. These types of experiments are by their very nature long term, and should be initiated with other forest tree species. Somaclonal variation is a problem that must be addressed.

SUMMARY AND CONCLUSIONS

Literature and consultations were used to assess feasibility of altering physical and chemical properties of wood from species used in short rotation "energy plantations". The objective of such alteration was seen as improving efficiencies of processes for converting biomass into ethanol or gaseous or other liquid fuels. Opportunities for manipulation via both conventional selection and breeding and molecular or so-called biotechnological approaches were considered. Results are presented in three sections: Conventional Selection and Breeding, Biosynthetic Pathways, and Potential Applications of Molecular Genetics.

Traits vary in their impact on conversion efficiencies, and an assessment was made of relative importance: cellulose and lignin quantity/quality were considered most important, specific gravity and moisture quantity moderately important, bark quantity, extractives, and ash content of minor importance, and calorific value least important. Suitability for products other than energy was considered critical.

Survey results indicate opportunities for meaningful manipulation of most traits. Improvement in some traits, e.g., cellulose content and specific gravity, has already been demonstrated via conventional selection and breeding. Altering other important traits by these means is feasible provided that new and better estimates of genetic variation, heritabilities, genetic correlations, and genotype X environment interactions are forthcoming.

In many cases, however, routine modification on a usable scale lies some years in the future. This is especially true for manipulation via molecular genetics. Existing techniques for transferring foreign genes as well as confirming their expression and transmission are available. Information about the enzymes and genes underlying and controlling important traits, however, is insufficient for application of most molecular techniques in the near term. Continued, or preferably accelerated, research will be required before traits such as lignin quantity/quality can be altered at will, even though this constituent is the most vulnerable to attack via molecular genetics. Several molecular techniques, however, can be used to good effect in the near term. Antisense RNA techniques, e.g., could be developed for use in characterizing enzyme functions in lignin biosynthesis.

Prospects for and feasibilities of manipulation are detailed for individual traits in paragraphs on "recommendations" at the end of each major section. Suggestions concerning research needs and directions are also provided therein.

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APPENDIX

APPENDIX TABLE I. Representative values for selected wood and fiber properties[a]

Characteristic or property	Species									
	Aspen	Eastern cottonwood	Black cottonwood	Black willow	Silver maple	Red maple	Sycamore	Green ash	Sweetgum	Red alder
Specific gravity[b]	0.35	0.37	0.32	0.34	0.44	0.49	0.46	0.56	0.44	0.37
Fiber length, mm	1.04	1.00	1.38	1.10	0.76	0.82	1.70	1.27	1.70	1.20
Fiber diameter, μm	10-27	25-40	23-40	16-32	16-30	16-30	20-36	(14-26)	20-40	16-40
Volume of:										
Fiber, %	55	–[c]	–	54	67	69	29	(57)	27	–
Vessels, %	34	–	–	38	21	18	52	(15)	55	–
Rays, %	11	–	–	7	12	13	19	(14)	18	–
Lignin, %	18.2	22.8	21.4	–	–	22.9	24.0	(26)	22.8	23.9
α-cellulose, %	50.2	47.0	49.1	–	–	47.2	(43.0)	–	44.4	–
Hemicellulose, %	21.2	–	–	–	–	29.3	(27.2)	–	30.6	–
Total pentosans, %	17.5	18.5	19.2	–	20.4	19.6	–	–	18.5	22.8
Extractives:										
Alcohol-benzene, %	2.4	1.4	2.7	2.6	3.5	2.5	2.2	5.0	3.1	–
Hot water, %	2.3	2.0	2.6	–	–	4.4	–	–	4.6	1.6
Calorific value, k cal/kg	3215	–	–	–	–	3215	–	4275	4260	4445

[a]All values from Isenberg 1981, except for those in (). Latter were secured from a variety of sources cited elsewhere in text.
[b]Green volume basis.
[c]Indicates data not available.

APPENDIX TABLE II. Representative values for selected bark properties[a]

Characteristic or property	Species									
	Aspen	Eastern cottonwood	Black cottonwood	Black willow	Silver maple	Red maple	Sycamore	Green ash	Sweetgum	Red alder
Specific gravity[b]	0.50	0.31	0.40	0.34	0.57	0.60	0.60	0.48	0.42	0.58
Fiber length, mm	1.0	1.0	–[d]	–	–	–	–	–	1.05	–
Fiber diameter, μm	18-20	20	20-25	–	–	–	–	–	20-30	–
Fibrous yield, %[c]	8	9	12	–	6	12	–	13	5	–
Extractives:										
Alcohol-benzene, %	15.0	7.9	20.0	6.9	6.6	14.8	8.1	15.0	11.6	6.0
Hot water, %	4.7	4	–	4.8	–	16.0	3.6	–	8.2	3.7
Ash, %	4.0	6.2	5.0	6.2	3.6	5.2	6.4	6.5	8.1	4.0
Ca, %	1.9	2.5	1.1	1.8	0.6	1.5	3.0	1.8	3.8	1.4
Si, %	0.03	0.18	0.8	0.08	0.18	0.37	0.06	0.12	1.41	0.05
Calorific value, k cal/kg	4685	–	–	3985	4645	4220	4115	4150	4140	4415

[a]All values from Isenberg 1981, except for those in (). Latter were secured from multiple sources.
[b]Green volume basis.
[c]Fibrous yield after pulping to solids content of 30-35%.
[d]– indicates data not available.

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