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THE EFFECT OF MECHANICAL PERTURBATION ON THE
CONDUCTIVITY, MECHANICAL STRENGTH, AND
ABOVEGROUND BIOMASS OF SEVEN HYBRID POPLARS

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THE EFFECT OF MECHANICAL PERTURBATION ON THE CONDUCTIVITY, MECHANICAL STRENGTH, AND ABOVEGROUND BIOMASS OF SEVEN HYBRID POPLARS

By

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ABSTRACT

THE EFFECT OF MECHANICAL PERTURBATION ON THE CONDUCTIVITY, MECHANICAL STRENGTH, AND ABOVEGROUND BIOMASS OF SEVEN HYBRID POPLARS

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To mimic the wind-induced back and forth motion of plants under greenhouse conditions, mechanical perturbation, MP, (20 flexures per day) was applied to stems of sapling poplar clones for 70-90 days. This study quantifies the effect of MP on the wood properties of hydraulic conductivity and mechanical strength, and total aboveground biomass for seven hybrids of a cross between *Populus trichocarpa* (black cottonwood) and *P. deltoides* (eastern cottonwood). Mechanical perturbation of stems was expected to decrease hydraulic conductivity (k_h) and aboveground biomass, and increase mechanical strength. Mechanical perturbation significantly decreased specific conductivity (k_s or k_h per conductive xylem cross sectional area) and aboveground biomass. Flexural rigidity (EI), wood density, and xylem transverse area at point of flexure increased while modulus of elasticity (MOE) and modulus of rupture (MOR) decreased in most of the mechanically perturbed stems. Surprisingly, $k_{h\ max}$ was positively correlated with EI ($P<0.0001$, $R^2=0.507$) with some hybrids having both great mechanical strength and water conduction (e.g. Hybrid 19-61) while others were deficient in both properties. Similarly, both k_s and MOE were positively correlated with percent vessel lumen area ($P<0.0001$ and $P=0.002$, respectively).

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INTRODUCTION

In recent years poplar plantations have emerged as a new form of tree production, occupying approximately 15,000 ha in the region between southern Oregon and British Columbia (Heilman et al. 1995). Washington State University Hybrid Poplar Research Program reports there are currently 40,470 ha of hybrid poplar plantations in the Pacific Northwest (Johnson and Ekuan 2002). *Populus trichocarpa* (black cottonwood) was successfully hybridized with *P. deltoides* (eastern cottonwood) in 1971 at Washington State University (Johnson 2000). Some of the first hybrids outgrew their parents by 20-30% due to heterosis (Stettler et al. 1996). Heterosis is the increased vigor or superior quality of particular traits arising from crossbreeding genetically different plants or animals (Allard 1960). Heterosis drives hybrid poplar breeding.

Hybrid poplars are a source of a wide range of wood products as well as environmental protection and improvement (historically, windbreaks and erosion control). Presently, the majority of plantations in the Pacific Northwest are used as fiber for the pulp and paper industry (Zsuffa et al. 1996). Interest in using hybrid poplar for other wood products has increased over the years. Wood from hybrid poplar is now utilized in products such as lumber, veneer and plywood, composite panels (e.g. particleboard, fiberboard, waferboard), structural composite lumber (e.g. parallel strand lumber, laminated strand lumber), pallets, furniture components, fruit baskets, containers, and chopsticks (Balatinecz and Kretschmann 2001). In some cases hybrid poplars are even used as biomass for energy, carbon sequestration, and phytoremediation of environmental problems (Kuhn and Nuss 2000).

Populus trees establish easily and have rapid juvenile growth compared to other tree species, advantages important to their successful use as windbreaks. Hybrids derived from species adapted to riparian areas act as vegetation filters for use in stream stabilization and in riparian buffers to protect and improve water quality, taking up excess nutrients and chemicals moving from adjacent farmlands (Johnson 2000). For scientists, *Populus* has rapidly become a model organism for the study of tree biology through the coordinated collection and distribution of clones supported by industry/government/academic partnerships, and funding from multi-agency grants (Bradshaw et al. 2000). In fact, one of the standard materials that is most widely used by researchers is a three-generation pedigree developed at the University of Washington which includes one parent each of *P. trichocarpa* and *P. deltoides*, two of their F_1 offspring, and an F_2 family ($N = 379$) derived from them (Stettler and Bradshaw 1996). Additionally, *Populus* has become a model organism for the study of tree biology because our current understanding of secondary tissue development in woody angiosperms is based on the detailed analysis of vascular development within the stem of *P. deltoides* (Larson and Isebrands 1974, Larson 1979, Richards and Larson 1982, Meicenheimer and Larson 1983).

Most *Populus* plantations are established on former agricultural lands, which are often unsheltered and windy (Harrington and DeBell 1996). At harvest (6-8 yrs old), commercial clones of hybrid poplars grown under intensive culture in plantations average 21 m in height and 25 cm in diameter (Johnson 2000). Understanding the effect of wind stress on the wood properties of hybrid poplar is important for continuing to select hybrids that improve the production of woody biomass for energy and fiber.

In nature, trees are constantly subjected to winds that apply external loads or mechanical tension to stems and branches (Mattheck 1991). Temporary bending of tree stems in response to wind stress or MP is considered an elastic strain and subsequently induces the formation of flexure wood (Telewski 1989, 1995). In this case, reaction wood is not formed because the displacement of the stem from its original vertical position is not permanent. Instead, the stem oscillates back and forth, alternating between compressional and tensional strains, until the motion diminishes completely and the stem returns to the vertical position.

The most obvious effect of wind on trees in the field is decreased height growth and increased radial growth. Jacobs (1954) found that free-swaying *Pinus radiata* (Monterey pine) grew more in diameter over the lower part of the trunk than trees held by guy wires to prevent wind sway and had increased eccentric trunk development along the line of the main winds. In the greenhouse, using wind generated by oscillating electric fans, Larson (1965) found that *Larix laricina* (tamarack) held upright with guy wires grew much taller and thinner than their unrestricted neighbors.

Telewski and Jaffe (1986a) compared *Abies fraseri* (Fraser fir) growing on windward and leeward sites located along the Roan High Bluff ridge crest of Roan Mountain, NC. They found significant changes in growth of trees on the windward sites: longitudinal growth of seedling internodes was inhibited, radial growth was reduced along both major axes in the young leaders exposed to prevailing winds, and the cross-sections of both saplings and mature trees were substantially elliptical. In another field study, Cordero (1999) compared growth of *Cecropia schreberiana* saplings in two different wind regimes (mean wind speed at windy sites was 32 times greater than at protected sites) in an elfin cloud forest. He found that wind exposure caused significant reductions in height, basal diameter, leaf area, and total biomass.

In the Cordero (1999) study, decreased basal diameter of wind-exposed stems was the opposite of expected. Two observations may explain this discrepancy. Cordero (1999) reported a significant increase in root to shoot ratio or greater investment in anchorage in response to wind treatment. Decreased aboveground biomass may have been great enough to reduce radial growth of wind-exposed stems. Additionally, the relative size of the pith cavity in wind-protected stems and wind-exposed stems may have affected the relative diameter of stems in the two treatments. *C. schreberiana* stems at the windy sites probably had a greater proportion of parenchymatous tissues (less fibers and vascular tissues) and a relatively greater pith, whereas the larger stems of the wind-protected plants probably had greater proportions of vascular and supportive tissues, and less pith (Cordero 1999).

Damage from a windstorm at a three-year-old *Populus* research trial revealed that clonal differences in susceptibility to wind toppling or leaning were associated with both above- and below-ground characteristics (Harrington and DeBell 1996). For example, Hybrid 47-174, the clone most resistant to wind toppling (wind tolerant), had the lowest aboveground woody biomass per unit of cross-sectional root area and highest rates of lower stem taper. In comparison, Hybrid 11-11, the clone most susceptible to wind damage (wind intolerant), had the highest aboveground woody biomass per mean root area and lowest rates of lower stem taper.

Various plant responses to wind occur simultaneously, making it difficult to isolate their individual effects on plant growth (Biddington 1986, Telewski 1995). The wind-induced back and forth motion of plants has been simulated in the laboratory by mechanical perturbation (MP) including rubbing, vibrating, shaking, or flexing of stems (Neel and Harris 1971, Jaffe 1976, Telewski and Jaffe 1986a, Niklas 1998, Cipollini 1999, Pruyn et al. 2000). Shaking of *Liquidambar styraciflua* (sweet gum) trunks resulted in decreased internode length, fewer nodes, and a 20% decrease in height growth (Neel and Harris 1971). Jaffe (1976) found that rubbing the first true internode of *Phaseolus vulgaris* (beans) retarded stem elongation (due to decreased internode length) and flower production, but increased internode diameter. Flexure of *Pinus taeda* (loblolly pine) inhibited stem and needle elongation, bracing of branch nodes, and increased radial growth in the direction of MP (Telewski and Jaffe 1986b). The vibration of *Capsella bursa-pastoris* (shepard's purse) resulted in more massive root systems and less massive vegetative shoots, reduced dry weight of reproductive structures at maturity, delayed formation of first mature flower and fruit, and accelerated onset of plant senescence (Niklas 1998). Cipollini (1999) found that flexing *Brassica napus* (Cruciferae) reduced plant height and root mass; increased stem diameter while decreasing pith diameter; delayed both cotyledon senescence and date of anthesis; reduced flower number, leaf area, and aboveground biomass. Pruyn et al. (2000) found that flexing *Populus* hybrids increased radial growth especially in the direction of MP and decreased the height to diameter growth ratio.

The morphological and physiological responses of plants to MP are collectively termed thigmomorphogenesis (Jaffe 1976). Several authors have reviewed the effects of thigmomorphogenesis on plants: Biddington (1986), Jaffe and Forbes (1993), Telewski (1995), and Jaffe et al. (2002). The total effect of flexure stress on tree growth and development (thigmomorphogenesis) results in a more compact growth form including greater stem taper, shorter branches, and smaller leaves (Telewski 1995).

Mechanical perturbation of stems in the laboratory produces growth characteristics that mimic the growth of plants exposed to wind in the field. In 1980, Jaffe published a photograph showing that hand-rubbed beans grown in the greenhouse and untreated beans exposed to wind were stunted to a similar extent when compared to untreated controls grown in the greenhouse. Telewski and Jaffe (1986a) reported that flexure of greenhouse-grown *A. fraseri* reduced extension growth, increased stem diameter, and produced an elliptical stem cross-section, characteristics that were all present in field grown *A. fraseri* exposed to high velocity winds.

Clearly wind stress affects the development of wood (secondary xylem) in trees (Jacobs 1954, Larson 1965, Telewski and Jaffe 1986a, Pruyn et al. 2000). Since wood functions as mechanical support in trees, the mechanical properties of wood are also subject to change. Responses of both field-grown and greenhouse-grown *A. fraseri* to MP included increased flexural rigidity (EI) or a stronger stem, and decreased modulus of elasticity (MOE) or more flexible stem tissue (Telewski and Jaffe 1986a). Pruyn et al. (2000) reported similar results for two hybrid poplars: EI increased 31% and MOE decreased 14% in MP clones compared to controls. Flexural rigidity is a measure of the rigidity of the whole stem while MOE is a measure of the elasticity per unit stem cross-sectional area. Mechanical perturbation of *P. taeda* decreased flexibility and increased elasticity and plasticity of the stem (Telewski and Jaffe 1986b). Using a different approach, Holbrook and Putz (1989) examined the biomechanics of four-year-old *L. styraciflua* after the seedlings were prevented from wind sway for two years in the field.

They found that whole-tree flexibility (a parameter similar to EI) and MOE tended to decrease, although not significantly, in free swaying trees compared to constrained trees.

Many of the responses observed in wind-stressed or mechanically perturbed trees are examples of developmental acclimation, resulting in altered biomass allocation that enables trees to withstand bending or swaying stresses by reducing drag and increasing mechanical strength (Telewski and Jaffe 1986a). However, the function of wood is not solely mechanical support. A second important function of wood is the long distance transport of water from roots to leaves (water conduction).

In gymnosperms a single cell type, the tracheid, is responsible for both water conduction and mechanical support. Spicer and Gartner (2002) bent seedlings of *Pseudotsuga menziesii* (Douglas-fir) such that the angle of the stem was 25° or 50° from vertical during their entire fourth growing season to assess the impact of compression wood (CW) formation on whole-plant hydraulic properties. Compression wood is the type of reaction wood formed on the lower sides of inclined stems and branches of gymnosperms (Esau 1977). Spicer and Gartner (2002) found that %CW increased and specific conductivity (k_s, conductivity normalized for sapwood area) decreased in seedlings induced to form CW. Compression wood shares some of the characteristics of flexure wood: lower MOE, greater density, and shorter, narrower tracheids (Telewski and Jaffe 1986a, Timell 1986, Telewski 1989). Compression wood of *P. menziesii* is expected to have tracheids that are shorter and narrower, one explanation for the decrease in k_s. Telewski (1989) concluded that wood formed under the influence of flexural stress has a morphology and function more closely related to compression wood than normal wood despite several differences (i.e. flexure wood tracheids differ from CW tracheids in that they are not rounded with a thicker S_2 cell wall layer and intercellular spaces). For this reason, the formation of flexure wood in trees may also decrease k_s. Despite this tradeoff, Spicer and Gartner (2002) found that the water potential and stomatal conductance of inclined seedlings remained similar to controls indicating that even a severe reduction in stem k_s caused by compression wood had little impact on leaf-level processes.

The question of a trade-off between the functions of mechanical support and water conduction may also be applied to woody angiosperms. With increasing specialization, trees with conducting elements that were more efficient in conduction than in support evolved (Esau 1977). As a result, two different cell types in woody angiosperms are specialized to function in mechanical support (fibers) and water conduction (vessel elements). For example the xylem of poplar is a complex combination of cell types including vessel elements (28-34%), fibers (53-60%), and axial (0.1-0.3%) and ray parenchyma (11-14%) (Balatinecz and Kretschmann 2001).

Preliminary work on *Quercus ilex* (holly oak) showed that inclined seedlings had the same k_s as vertical controls, suggesting an effective decoupling of transport and support functions in angiosperms (Spicer and Gartner 2002). In the case of these inclined seedlings, tension wood, the type of reaction wood formed on the upper sides of inclined stems and branches of arboreal dicotyledons, is expected to form and not flexure wood. The anatomical characteristics of flexure wood in angiosperms are not as extensively documented as in gymnosperms, however, MP of *L. styraciflua* showed a decrease in fiber length and that vessel members decreased in length and diameter (Neel and Harris 1971). In addition, Pruyn (1997) showed that vessel diameter and frequency decreased in the flexure wood of two hybrid poplars. Given that vessel diameter and frequency decreased, conductivity is likely to decrease in woody angiosperm stems subjected to MP.

Despite this information, the relationship between the mechanical strength of wood and conductivity has received little study. In one study, Wagner et al. (1998) reported an inverse relationship between the efficiency of water transport and the mechanical strength of wood when comparing closely related chaparral shrubs growing in the same habitat. Greater vessel lumen area and two-fold greater ks corresponded to lower stem density, MOE, and modulus of rupture (MOR). However, in another study the lack of correlation among xylem specific conductivity and vessel diameter with MOR and xylem implosion resistance was reported for 22 species of chaparral shrubs, suggesting the mechanical properties of chaparral xylem have little impact on water transport efficiency (Davis et al. 2002).

Similarly, Woodrum et al. (2003) assessed hydraulic conductivity and mechanical properties of lateral, woody branches of five species of *Acer* (Aceraceae) and found no trade-off between $k_{s\,max}$ and MOE or MOR. However, fiber lumen diameter was inversely correlated to MOE and MOR suggesting that differences in mechanical properties may be due to fiber lumen differences that do not influence the efficient transport of water.

The relationship between conductivity and mechanical support, the two primary functions of wood, must be considered in order to optimally select hybrid poplar clones that continue to improve the production of woody biomass for energy and fiber. Poplar hybrids continue to be developed via selection in breeding programs and by genetic engineering. Studying the effect of MP on conductive capacity, mechanical strength, and aboveground biomass of greenhouse grown hybrid poplars is one step towards understanding the dynamic effect of wind stress on hybrid poplars grown in plantation.

The present study quantifies the effect of MP on hydraulic conductivity and mechanical strength, two of the primary functions of wood, and aboveground biomass for seven hybrids of a cross between *P. trichocarpa* and *P. deltoides*. This study expands upon an investigation conducted by Pruyn et al. (2000) in which the mechanical responses of two poplar hybrids (Hybrid 11-11, characterized as wind intolerant; 47-174, characterized as wind tolerant) subjected to MP were reported. Mechanical perturbation increased EI and decreased MOE, in effect reducing susceptibility of both clones to wind damage. In the present study, one of the hybrids, Hybrid 19-61, is a known tri/aneuploid (Bradshaw and Stettler 1993) while the other six hybrids are diploid. Attractive features of triploids have been their greater volume growth and longer, wider fibers (Stettler et al. 1996). Differences in conductivity, mechanical properties, and aboveground biomass among hybrids are reported. Thin cross-sections of stems are analyzed to determine whether there is an anatomical explanation for differences in conductivity and mechanical properties due to MP. Given current knowledge, the following predictions are made for greenhouse grown clones subjected to MP or control treatments:

1. Mechanical perturbation will alter biomass allocation of all clones, resulting in decreased height growth of the leader stems, increased radial growth of stems at point of flexure, and reduced leaf area.

2. Mechanical perturbation will decrease conductivity, increase mechanical strength, and decrease aboveground biomass of all clones.

3. Different hybrids will vary significantly in conductivity, mechanical properties, and aboveground biomass allocation.

4. Conductivity will be inversely proportional to mechanical strength of controls and clones subjected to MP.

5. Aboveground biomass will be proportional to conductivity and inversely proportional to mechanical strength of controls and clones subjected to MP.

6. Percent vessel lumen area will be positively correlated with conductivity.

MATERIALS AND METHODS

Culture of the Plants

Seven F1 hybrids of *Populus trichocarpa* x *P. deltoides* (T x D) were used in this study: 11-11, 11-5, 46-158, 49-177, 47-174, 55-260, and 19-61. These hybrids were originally developed as part of a joint hybrid poplar breeding program between the University of Washington and Washington State University. Hybrids 11-11 and 47-174 have been planted extensively in commercial plantations in western Oregon and Washington (DeBell et al. 2002). Stem cuttings of the trees came from several sources: the breeding program's field station in Puyallup, Washington, the USDA Forest Service in Olympia, Washington, and the Fort James Corporation. These hybrids have been cultivated at the Plant Science Greenhouses at Michigan State University since 1994, with photoperiod ranging from 12 to 14 hr and diurnal temperature maintained at approximately 25°C.

In December 2001 all of the trees were cut back to force new shoot growth. In January 2002 leader stems of uniform height and stem width were selected on all trees. Each leader stem was then staked. The trees were grown for 4 wk before treatment began.

Thirty trees of each hybrid were arranged in a complete randomized block design on five benches in the greenhouse. Fifteen trees of each hybrid were mechanically perturbed (20 flexures back and forth along the same plane) once daily from February 18 until May 8, 2002. The remaining 15 were controls (without mechanical perturbation). A location just above the fourth node from the base of the stem was designated as the point of flexure. The point of flexure is the place on the stem that is grasped with the thumb and forefinger of one hand while the other hand is used to flex the stem by grasping it approximately 10 cm above the flexure point. Flexing did not exceed 45°.

Fertilizer (20-20-20 N-P-K, 7.5 g L^{-1}) was applied to each individual tree three times between December 2001 and April 2002. During the experiment, Mavrik® (0.6 ml L^{-1} H_20) was applied once and Sanmite® (0.3 ml L^{-1} H_20) twice to control spider mites. To lower soil pH, 1.0 L of a sulfuric acid solution (0.1 ml H_2SO_4 L^{-1} H_20) was applied to each pot in February and March 2002.

Before and after the experiment, the height of the leader stem and diameter of the stem at the point of flexure were recorded. Diameter was measured with a digital caliper sensitive to 0.1 mm, in the direction of flexure (parallel to flexure) as well as perpendicular to flexure.

Hydraulic Conductivity

Sampling was completed over a 3 wk period, April 18 to May 8, 2002. A single stem segment was taken from each healthy tree (166 of 210 trees sampled). Using plastic water-filled bags to wrap around the stem, the stem was cut 15 cm below the point of flexure while locally submerged, to prevent the introduction of air embolism and re-cut 20 cm above the point of flexure while under water. The submerged stem segments were transported to the laboratory in a bucket of water. The stem portion distal to the cut segment was prepared for measurement of leaf area and oven dry weight.

To ensure a fresh-cut surface, the stem segments were re-cut under water in the laboratory such that the point of flexure was 5 cm above the base of the segment and 10 cm below the top. Hydraulic conductivity per unit pressure gradient (k_h, kg m MPa^{-1} s^{-1}) was measured as described by Sperry et al. (1988). To discourage microbial growth, degassed 10 mMol citric acid was run through a 0.2 μm Gelman filter and allowed to flow via gravity from a known height through tubing connected to the distal end of the stem segment. Volumetric flow rate was determined by recording the time required for 0.1 g of citric acid to flow through the stem segment and collect in a beaker on an analytic balance (model BP 1215, Sartorius AG Gottingen Germany).

Emboli were removed from the stem segments via perfusion with citric acid at 175 kPa for 15 min. Hydraulic conductivity measurements and perfusion were alternated until the rate of flow remained constant (less than a 10% difference observed) between consecutive trials for a given stem segment. Conductive vessels were stained using 0.5% Crystal Violet immediately following measurements of conductivity. Stem segments were stored in plastic bags and refrigerated until mechanical properties were measured 2 wks later.

Hydraulic conductivity (initial and maximum) were calculated using the equation:

$k_h = F/(dP/dx)$ (Tyree and Ewers 1991)

where F is the water flux (kg s^{-1}) and dP/dx is the pressure difference causing the flow (MPa m^{-1}). Specific conductivity was calculated as described by Tyree and Ewers (1991) using the equation:

$k_s = k_{h\ max} / A_s$

where A_s is the conductive xylem cross-section area (m^2). The degree of embolism in the stem segment was calculated using the initial conductivity as a percentage of the maximum after emboli were removed via the equation:

$$\% \text{ embolism} = \{(k_{h\,max} - k_{h\,initial}) / k_{h\,max}\} * 100\%$$

A subsample of eight trees of each hybrid, four control and four treatment, were randomly selected to measure the leaf area distal to each segment using a LI-COR area meter (model LI-3100, LI-COR Inc., Lincoln, NE). The leaves of all trees were oven dried at 45°C to a constant weight. Leaf area of the remaining trees was estimated by linear regression using equations derived from plots of leaf area and oven dry leaf weight. The total leaf area distal to the segment was calculated for each segment and used to calculate leaf specific conductivity using the equation:

$$LSC = k_h / A_l \qquad \text{(Tyree and Ewers 1991)}$$

where A_l is the total leaf area (m^2). In cases where leaves were present on the 15 cm segment, 50% of this leaf area was included in the LSC calculation (Ewers et al. 1989). The Huber value, defined as:

$$HV = A_s / A_l \qquad \text{(Tyree and Ewers 1991)}$$

was also calculated so any difference in LSC could be attributed to differences inherent to the wood.

Mechanical Properties

The 15 cm stem segments used to measure hydraulic conductivity were also used to measure mechanical properties: flexural rigidity (EI, N mm^2), second moment of cross-sectional area (I, mm^4), modulus of elasticity (MOE, N mm^{-2}), modulus of rupture (MOR, N mm^{-2}), and the specific gravity of wood at green volume (sp gr). Segments were kept at approximately 10°C until testing with an Instron Universal Machine (model 4202, Instron Corporation, Canton MA) at the Department of Agricultural Engineering at

Michigan State University. A four-point bending test with a compression load cell of 500 N was conducted as described by Pruyn et al. (2000). The span length (L), the distance between the two supported ends, was 135 mm. The distance (a) between one supported end and the nearest load (F) was equal to 45 mm. The load was applied at two points along the span length at a crosshead speed of 20 mm/min. Stress versus strain data were collected every 0.1 mm using a computer with Cy4200 software networked to the Instron.

Stem segments were deflected until the load reached a maximum value, known as the critical strain, reaching the limit of the elastic range (Spatz and Bruechert 2000). Stem segments did not rupture under stress due to the juvenility of the wood. However, after testing the stem segments did not return to their original shape either, indicating the elastic limit had been exceeded. The modulus of rupture (MOR) was estimated using the load value at the asymptote of the curve and the equation:

$\text{MOR} = (F_{max} * a * R_{major})/I$ (modified from Ugural 1991)

where F_{max} is the load at stem failure, R_{major} is the major radius of the stem segment minus the pith, and I is the second moment of cross sectional area for a hollow ellipse:

$I = (\pi * R_{major} \cdot R_{minor}^3)/4$ (Wainwright et al. 1982)

Maximum and minimum stem diameters were measured with a digital caliper at three points along the stem segment (at the center and 2 cm from each end) to determine mean maximum and minimum radii, R_{major} and R_{minor}, as stems were elliptical in cross-section. To calculate the second moment of cross sectional area (I), pith radius was measured at the center of the stem segment, raised to the 4th power, and subtracted from $R_{major} * R_{minor}^3$.

Flexural rigidity (EI) was calculated using the slope (F/V) of the linear portion (elastic part) of the curve and the equation:

$EI = (F/V)(a^2/12)(3L-4a)$ (modified from Gere and Timoshenko 1997)

Flexural rigidity was divided by second moment of cross sectional area to calculate the modulus of elasticity (MOE) (Pruyn et al. 2000).

Specific gravity (sp gr) of wood at green volume was calculated using the equation given by Panshin and de Zeeuw (1980): sp gr = (oven dry weight of wood)/(weight of displaced volume of water using green wood). A subsample from each segment was taken by cutting at the point of flexure and 3.5 cm above the point of flexure. Stem diameter, xylem diameter, and pith diameter were measured with a digital caliper before the cortex was removed from each subsample. Each subsample was submerged in a 10 ml graduated cylinder of distilled water and the displacement of water was recorded. The subsamples were oven dried at 45°C to a constant weight.

Aboveground Biomass

Stems and leaves from the apex of each tree to 5 cm below the point of flexure were oven dried at 45°C to a constant weight.

Stem Cross-Sections

Sixteen stems of Hybrids 46-158 and 19-61 (eight control and eight MP) were used for analysis of anatomical differences due to flexure treatment. Hybrids 46-148 and 19-61 were chosen because their mean k_s and MOE showed the greatest response to flexure treatment. Additionally, Hybrid 46-148 is known to be diploid while Hybrid 19-61 is triploid.

Transverse sections were taken approximately 3.5 cm above the point of flexure on each stem segment. Thin sections (approximately 40 μm thick) were made using a sliding microtome. Sections were subjected to a dehydration series of ethanol and xylene (modified from Johansen 1940) and then mounted on slides using Permount.

A light microscope interfaced with a CCD video camera and multi-scan analog monitor was used to analyze the sections. NIH Image 1.5 software was used to measure vessel lumen area per unit area of wood. Two areas were chosen on both sides of the pith along the same axis in all stems. In mechanically perturbed stems this axis was in the direction of flexure. The total area per stem scanned was 0.240 mm^2. Vessel lumen areas of each stem were used to calculate percent vessel lumen area, average and maximum vessel lumen diameter, hydraulic diameter, and vessel frequency. Hydraulic diameter was calculated using the equation:

$HD = \Sigma d^5 / \Sigma d^4$ (Sperry et al. 1994)

where d is the diameter of the vessel lumen.

Data Analysis

All data were analyzed using SAS Version 8.1. Analysis of variance for seven hybrids and two treatments (control and MP) was conducted for each variable using two-way ANOVAs to show the main effect of each factor. Tukey-Kramer was used for multiple comparisons among means of hybrids to identify which hybrids were significantly different from each other. The specific effect of treatment (control and MP) within each hybrid was conducted by slicing to identify which hybrids had a significant difference between control and MP. Some variables were transformed before analysis of variance because the data were not normally distributed according to at least 3 out of 4 tests for normality (Shapiro-Wilkinson, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling). Variables transformed using square root were leaf area, $k_{h\ max}$, k_s, LSC, aboveground biomass, sp gr, EI, and MOE. HV and $k_{h\ initial}$ were transformed using $\log_{10}$. Regression analysis was used to determine correlations between variables. Excel2000 was used to graph means and plot trendlines.

RESULTS

General Morphology

Multiple comparisons among means showed only Hybrids 11-5 and 47-174 differed significantly ($P=0.01$) with respect to height growth (Table 1). Hybrid 47-174 had greater height growth (125.5 ± 7.0 cm) than 11-5 (90.6 ± 7.0 cm). None of the hybrids differed significantly in the change of transverse area of stem at point of flexure or leaf area distal to stem segments. Hybrid 46-158 had the greatest Huber value.

Mechanical perturbation had a significant effect on several morphological characteristics (Table 1). It decreased the mean height growth of all hybrids ($P<0.0001$) and increased the change in transverse area of stem at point of flexure ($P<0.0001$). With the exception of Hybrid 49-177, leaf area distal to stem segments generally decreased ($P=0.04$) when clones were mechanically perturbed. Huber value ($P<0.0001$) increased in all hybrids with clones subjected to MP.

Hydraulic Conductivity

Multiple comparisons among means showed some hybrids inherently differed in conductive capacity on a whole stem basis, $k_{h\ \mathrm{initial}}$ and $k_{h\ \mathrm{max}}$, or on a tissue area basis, k_s (Table 2). Hybrid 19-61, a known triploid, had significantly higher $k_{h\ \mathrm{initial}}$, $k_{h\ \mathrm{max}}$, and k_s than several of the diploid hybrids. Hybrids 47-174 and 19-61 had lower percent embolism than Hybrids 11-11, 11-5, and 46-158 ($P<0.0001$). Hybrid 47-174 had the lowest LSC while LSC in Hybrids 46-158, 49-177, and 19-61 were significantly greater.

Mechanical perturbation of clones significantly decreased k_s ($P<0.0001$) when compared to controls (Table 2). Mechanical perturbation did not have a significant effect on $k_{h\ initial}$, $k_{h\ max}$, k_s, percent embolism, or LSC. However, $k_{h\ max}$ tended to decrease in mechanically perturbed clones with the exception of Hybrid 49-177.

Mechanical Properties

Mechanical properties on a whole stem basis (EI) and a tissue area basis (MOE, MOR) are shown in Table 3. Multiple comparisons among means showed that some hybrids differed significantly. Hybrid 19-61 had the highest EI, while EI of 49-177 was significantly lower ($P=0.04$). Hybrid 19-61 had the greatest MOE and MOR. Compared to Hybrid 19-61, MOE of Hybrids 11-11, 46-158, and 49-177 were significantly lower as was MOR of Hybrids 11-5, 46-158, 49-177, and 55-260. Hybrids with low mean specific gravity included 11-5 and 49-177 while Hybrids 47-174 and 19-61 had significantly greater wood specific gravity. Second moment of cross sectional area (I) did not significantly differ among hybrids.

Flexural rigidity ($P=0.01$) increased in clones subjected to MP, except in Hybrid 47-174. MOE ($P<0.0001$) and MOR ($P=0.0006$) decreased with MP with one exception: MOR of Hybrid 49-177 was not affected by flexure (80.14 ± 4.32 N mm^{-2} for control and 80.34 ± 2.82 N mm^{-2} for MP). Wood specific gravity at green volume significantly increased ($P<0.0001$) in clones subjected to MP compared to controls. Mechanical perturbation significantly increased second moment of cross sectional area ($P<0.0001$).

Aboveground Biomass

Statistically, all seven hybrids were similar in aboveground biomass accumulation (Table 1). However, clones of all hybrids showed decreased aboveground biomass accumulation when subjected to MP (P=0.04).

Stem Cross-Sections

Analysis of vessels from stem cross-sections of Hybrids 46-158 and 19-61 (hybrids that showed the most pronounced response to MP in terms of k_s and MOE) are shown in Table 4. When comparing hybrid means, Hybrid 46-158 had lower vessel lumen area than 19-61 (P=0.0003). Hybrid 46-158 had higher VD_{avg} than Hybrid 19-61 (P<0.0001). There was no significant difference between hybrids for VD_{max}, HD, and vessel frequency.

Mechanical perturbation of clones decreased vessel lumen area, VD_{avg}, VD_{max}, HD, and vessel frequency compared to controls for both hybrids; however, there was a significant Hybrid x MP interaction for each variable except vessel frequency. Mechanical perturbation decreased lumen area by 38% for Hybrid 46-158 and only 22% for Hybrid 19-61. Mechanical perturbation decreased VD_{avg} by 49% for Hybrid 46-158 and only 19% for Hybrid 19-61, but the average vessel diameters were similar for mechanically perturbed clones of both hybrids. Mechanical perturbation decreased VD_{max} by 31% for Hybrid 46-158 and only 9% for Hybrid 19-61. Maximum vessel diameters of mechanically perturbed clones of Hybrid 19-61 were 23% wider than Hybrid 46-158. Mechanical perturbation decreased HD by 29% for Hybrid 46-158 and only 6% for Hybrid 19-61. Hydraulic diameter of mechanically perturbed clones of

Hybrid 19-61 was 22% greater than Hybrid 46-158. Mechanical perturbation of clones significantly decreased (P=0.05) vessel frequency of both hybrids.

Correlations

Contrary to the hypothesis, conductivity was not inversely proportional to mechanical strength but, instead, positively correlated to mechanical strength. At the whole stem level, $k_{h\ max}$ showed a significant positive correlation with EI (P<0.0001, Figure 1). This relationship was also evident at a per unit xylem level as k_s was positively correlated with MOE (P=0.02, Figure 2) and MOR (P<0.0001, Figure 3). When Hybrid 19-61, the triploid hybrid that exhibited great conductive capacity and mechanical strength, was removed from the regression analysis, the correlation between k_{hmax} and EI (P<0.0001) remained significantly positive, as did the correlation between k_s and MOR (P=0.0004). However, when Hybrid 19-61 was removed from regression analysis for k_s and MOE, no correlation existed (P=0.4).

Aboveground biomass was positively correlated with $k_{h\ max}$ (P<0.0001, Figure 4), EI (P<0.0001, Figure 5), and leaf area (P<0.0001, Figure 6). Modulus of rupture was positively correlated with MOE (P<0.0001, Figure 7) and wood specific gravity (P<0.0001, data not shown). There was a significant positive correlation between MOR and wood specific gravity of the controls (P=0.009, Figure 8) but this relationship was not observed in the clones subjected to MP. Modulus of elasticity was not correlated with wood specific gravity (P=0.4, R^2=0.0048, data not shown).

Analysis of vessel anatomy of Hybrids 46-158 and 19-61 showed k_s and vessel lumen area were positively correlated (P<0.0001, Figure 9) as were MOE and vessel lumen area (*P*=0.002, Figure 10). On the other hand, EI tended to decrease as vessel lumen area increased but this was not a significant negative correlation (*P*=0.9, R^2=0.0012, data not shown).

Specific conductivity tended to increase when vessel frequency increased but this was not a significant positive correlation (*P*=0.3, Figure 11); however, MOE and vessel frequency were positively correlated (*P*=0.001, Figure 12). Average vessel lumen diameter did not significantly correlate with k_s, $k_{h\ max}$, EI, MOE, or MOR (data not shown).

Table 1. General Morphology. Values are mean ± SE. Means in the same column with the same letter indicate hybrids are not significantly different ($P < 0.05$) by two-way ANOVA followed by multiple comparison among hybrid means. Asterisk (*) indicates MP was significantly different from control within each hybrid at $P < 0.05$ (** indicates significantly different at $P < 0.0001$). P_{MP} value is based on two-way ANOVA of treatment means. Δ Height = height after MP minus height before MP; Δ Transverse Area = transverse area of stem at point of flexure after MP minus transverse area before MP; Distal LA = leaf area distal to stem segment; Biomass = dry weight of aboveground biomass.

Hybrid	Treatment	N	Δ Height (cm)	Δ Transverse Area (mm^2)	Distal LA (m^2)	Huber value 10^{-5}	Biomass (g)
11-11	control	13	113.9 ± 7.1 ^{ab}	61.4 ± 5.0 ^{a}	0.832 ± 0.074 ^{a}	6.81 ± 0.449 ^{acde}	66.3 ± 6.9 ^{a}
	MP	13	76.8 ± 8.8 *	87.7 ± 7.1 *	0.756 ± 0.087	10.7 ± 1.17 *	54.7 ± 6.5
11-5	control	12	90.6 ± 7.0 ^{a}	51.2 ± 5.2 ^{a}	0.711 ± 0.112 ^{a}	7.31 ± 0.658 ^{abcde}	50.9 ± 8.4 ^{a}
	MP	13	71.3 ± 5.1	67.9 ± 7.8	0.563 ± 0.100	11.1 ± 0.972 *	35.7 ± 5.9
46-158	control	11	101.4 ± 13.7 ^{ab}	55.4 ± 9.2 ^{a}	0.601 ± 0.123 ^{a}	8.40 ± 0.515 ^{b}	56.8 ± 11.5 ^{a}
	MP	11	71.2 ± 7.0 *	88.7 ± 10.1 *	0.522 ± 0.094	14.5 ± 1.19 **	46.7 ± 8.3
49-177	control	12	104.4 ± 9.6 ^{ab}	48.6 ± 5.0 ^{a}	0.536 ± 0.042 ^{a}	8.08 ± 0.693 ^{abd}	49.2 ± 4.7 ^{a}
	MP	11	88.6 ± 5.2	73.2 ± 6.2 *	0.566 ± 0.102	11.5 ± 1.24 *	47.4 ± 6.4
47-174	control	11	125.5 ± 7.0 ^{b}	61.4 ± 6.8 ^{a}	0.809 ± 0.074 ^{a}	6.06 ± 0.222 ^{e}	66.7 ± 8.2 ^{a}
	MP	11	90.6 ± 8.2 *	75.0 ± 12.7	0.654 ± 0.078	7.71 ± 0.389	41.4 ± 6.4
55-260	control	11	107.6 ± 8.0 ^{ab}	52.2 ± 6.2 ^{a}	0.626 ± 0.096 ^{a}	7.78 ± 0.609 ^{ab}	48.6 ± 8.3 ^{a}
	MP	12	80.1 ± 8.6 *	82.0 ± 8.3 *	0.508 ± 0.080	13.8 ± 1.49 **	43.3 ± 6.8
19-61	control	13	96.4 ± 5.5 ^{ab}	58.5 ± 7.2 ^{a}	1.007 ± 0.173 ^{a}	5.91 ± 0.777 ^{de}	63.3 ± 8.7 ^{a}
	MP	12	78.8 ± 7.5	102.1 ± 13.1 **	0.720 ± 0.104	10.7 ± 0.837 **	55.7 ± 8.0
	P_{MP} value		P < 0.0001	P < 0.0001	P = 0.0302	P < 0.0001	P = 0.0373

Table 2. Hydraulic Conductivity. Values are mean ± SE. Means in the same column with the same letter indicate hybrids are not significantly different ($P < 0.05$) by two-way ANOVA followed by multiple comparison among hybrid means. Asterisk (*) indicates MP was significantly different from control within each hybrid at $P < 0.05$ (** indicates significantly different at $P < 0.0001$). P_{MP} value is based on two-way ANOVA of treatment means. NS = not significant; $k_{h\ initial}$ = initial conductivity; $k_{h\ max}$ = maximum conductivity; k_s = specific conductivity; LSC = leaf specific conductivity.

Hybrid	Treatment	N	$k_{h\ initial}$ 10^{-4} kg m MPa^{-1} s^{-1}	$k_{h\ max}$ 10^{-4} kg m MPa^{-1} s^{-1}	Embolism %	k_s 10^{-6} kg MPa^{-1} s^{-1} m^{-1}	LSC 10^{-4} kg MPa^{-1} s^{-1} m^{-1}
11-11	control	13	2.20 ± 0.492 ^{ab}	3.87 ± 0.526 ^{ab}	45.00 ± 4.62 ^{b}	7.40 ± 0.777 ^{ab}	4.72 ± 0.563 ^{ab}
	MP	13	2.14 ± 0.378	3.64 ± 0.454	50.94 ± 5.81	5.14 ± 0.382 *	5.17 ± 0.661
11-5	control	12	1.59 ± 0.328 ^{a}	3.27 ± 0.474 ^{ab}	42.34 ± 4.52 ^{b}	7.24 ± 0.677 ^{a}	4.85 ± 0.685 ^{ab}
	MP	13	1.85 ± 0.622	2.84 ± 0.713	54.10 ± 5.63	4.95 ± 0.654 *	4.89 ± 0.592
46-158	control	11	1.76 ± 0.511 ^{a}	3.35 ± 0.611 ^{ab}	53.73 ± 5.55 ^{b}	7.74 ± 0.690 ^{a}	6.17 ± 0.682 ^{b}
	MP	11	1.24 ± 0.377	2.42 ± 0.505	51.76 ± 5.82	3.62 ± 0.537 **	5.22 ± 0.959
49-177	control	12	1.49 ± 0.267 ^{ab}	2.70 ± 0.421 ^{ab}	34.25 ± 5.22 ^{ab}	6.51 ± 0.723 ^{ab}	5.91 ± 1.50 ^{b}
	MP	11	2.03 ± 0.345	2.99 ± 0.325	46.59 ± 5.12	5.88 ± 0.525	6.58 ± 0.987
47-174	control	11	2.30 ± 0.460 ^{ab}	3.10 ± 0.532 ^{a}	27.53 ± 7.10 ^{a}	6.38 ± 0.755 ^{a}	4.08 ± 0.767 ^{a}
	MP	11	1.79 ± 0.528	2.31 ± 0.557	26.70 ± 5.09	4.10 ± 0.584 *	3.07 ± 0.458
55-260	control	11	1.45 ± 0.359 ^{a}	2.47 ± 0.356 ^{a}	39.67 ± 6.01 ^{ab}	5.96 ± 0.735 ^{a}	4.45 ± 0.725 ^{ab}
	MP	12	1.44 ± 0.307	2.11 ± 0.382	48.62 ± 6.39	3.33 ± 0.461 *	4.25 ± 0.690
19-61	control	13	3.56 ± 0.532 ^{b}	5.08 ± 0.802 ^{b}	36.00 ± 5.78 ^{a}	10.60± 0.675 ^{b}	5.97 ± 0.978 ^{b}
	MP	12	2.80 ± 0.618	4.17 ± 0.694	27.58 ± 3.51	5.84 ± 0.531 **	5.65 ± 0.477
	P_{MP} value		NS	NS	NS	P < 0.0001	NS

Table 3. Mechanical Properties. Values are mean ± SE. Means in the same column with the same letter indicate hybrids are not significantly different ($P = 0.05$) by two-way ANOVA followed by multiple comparison among hybrid means. Asterisk (*) indicates MP was significantly different from control within each hybrid at $P < 0.05$ (** indicates significantly different at $P < 0.0001$). P_{MP} value is based on two-way ANOVA of treatment means. I = second moment of cross sectional area; EI = flexural rigidity; MOE = modulus of elasticity; MOR = modulus of rupture; sp gr = wood specific gravity at green volume.

Hybrid	Treatment	N	I (mm^4)	EI (10^3 N mm^2)	MOE (N mm^{-2})	MOR (N mm^{-2})	sp gr (g cm^{-3})
11-11	control	13	240 ± 27.8 ^{a}	641 ± 97.2 ^{ab}	2724 ± 241 ^{a}	90.4 ± 4.73 ^{abcd}	0.30 ± 0.0081 ^{ab}
	MP	13	420 ± 55.3 *	944 ± 114	2329 ± 151	85.7 ± 4.05	0.31 ± 0.0075
11-5	control	12	178 ± 30.4 ^{a}	526 ± 80.6 ^{ab}	3163 ± 222 ^{ab}	80.2 ± 2.42 ^{b}	0.28 ± 0.0069 ^{a}
	MP	13	252 ± 62.9	588 ± 129	2453 ± 122 *	73.2 ± 4.41	0.31 ± 0.0085 *
46-158	control	11	187 ± 49.5 ^{a}	561 ± 149 ^{ab}	3030 ± 298 ^{a}	88.6 ± 6.09 ^{abd}	0.29 ± 0.0098 ^{ab}
	MP	11	391 ± 85.4 *	818 ± 176	2148 ± 234 *	78.8 ± 5.55	0.34 ± 0.0119 *
49-177	control	12	141 ± 21.8 ^{a}	412 ± 64.9 ^{a}	2980 ± 193 ^{a}	80.1 ± 4.32 ^{abd}	0.27 ± 0.0101 ^{a}
	MP	11	241 ± 38.8	540 ± 79.4	2292 ± 105 *	80.3 ± 2.82	0.31 ± 0.0096 *
47-174	control	11	204 ± 35.9 ^{a}	627 ± 93.5 ^{ab}	3360 ± 222 ^{ab}	94.5 ± 2.54 ^{acd}	0.32 ± 0.0058 ^{b}
	MP	11	249 ± 66.4	621 ± 149	2812 ± 235	86.3 ± 4.29	0.33 ± 0.0061
55-260	control	11	163 ± 36.4 ^{a}	524 ± 86.7 ^{ab}	3564 ± 258 ^{ab}	89.5 ± 4.56 ^{ab}	0.29 ± 0.0083 ^{ab}
	MP	12	316 ± 63.6 *	702 ± 141	2321 ± 127 *	76.6 ± 4.30 *	0.33 ± 0.0089 *
19-61	control	13	211 ± 43.6 ^{a}	706 ± 109 ^{b}	4147 ± 392 ^{b}	102.7 ± 3.25 ^{c}	0.31 ± 0.0097 ^{b}
	MP	12	451 ± 90.0 *	1056 ± 180	2728 ± 240 **	90.0 ± 4.02 *	0.34 ± 0.0184 *
	P_{MP} value		P < 0.0001	P = 0.0145	P < 0.0001	P = 0.0006	P < 0.0001

Table 4. Stem Cross-Sections. Values are mean ± SE for vessel data obtained from the two hybrids that exhibited the greatest response to mechanical perturbation in terms of k_s and MOE. P-values are based on two-way ANOVA. Asterisk (*) indicates MP was significantly different from control within each hybrid at $P < 0.05$ (** indicates significantly different at $P < 0.0001$). NS = not significant; Lumen Area = vessel lumen area; VD_{avg} = average vessel lumen diameter; VD_{max} = maximum vessel lumen diameter; HD = hydraulic diameter; Vessel Freq = # of vessels mm^{-2}.

Hybrid	Treatment	N	Lumen Area %	VD_{avg} µm	VD_{max} µm	HD µm	Vessel Freq mm^{-2}
46-158	control	8	25.9 ± 0.7	44.7 ± 1.1	70.9 ± 1.8	56.9 ± 1.2	151.5 ± 6.1
	MP	8	16.1 ± 0.7 **	22.9 ± 2.7 **	49.2 ± 6.0 *	40.5 ± 4.8 *	135.9 ± 8.9
19-61	control	8	26.8 ± 0.8	26.6 ± 1.7	70.5 ± 2.1	55.6 ± 2.2	172.9 ± 20.0
	MP	8	21.0 ± 0.6 **	21.5 ± 0.7 *	64.3 ± 3.3	52.2 ± 2.4	138.0 ± 8.2
P_{hybrid} value			P = 0.0003	P < 0.0001	NS	NS	NS
P_{MP} value			P < 0.0001	P < 0.0001	P = 0.0008	P = 0.0024	P = 0.0460
$P_{interaction}$ value			P = 0.0081	P < 0.0001	P = 0.0439	P = 0.0346	NS

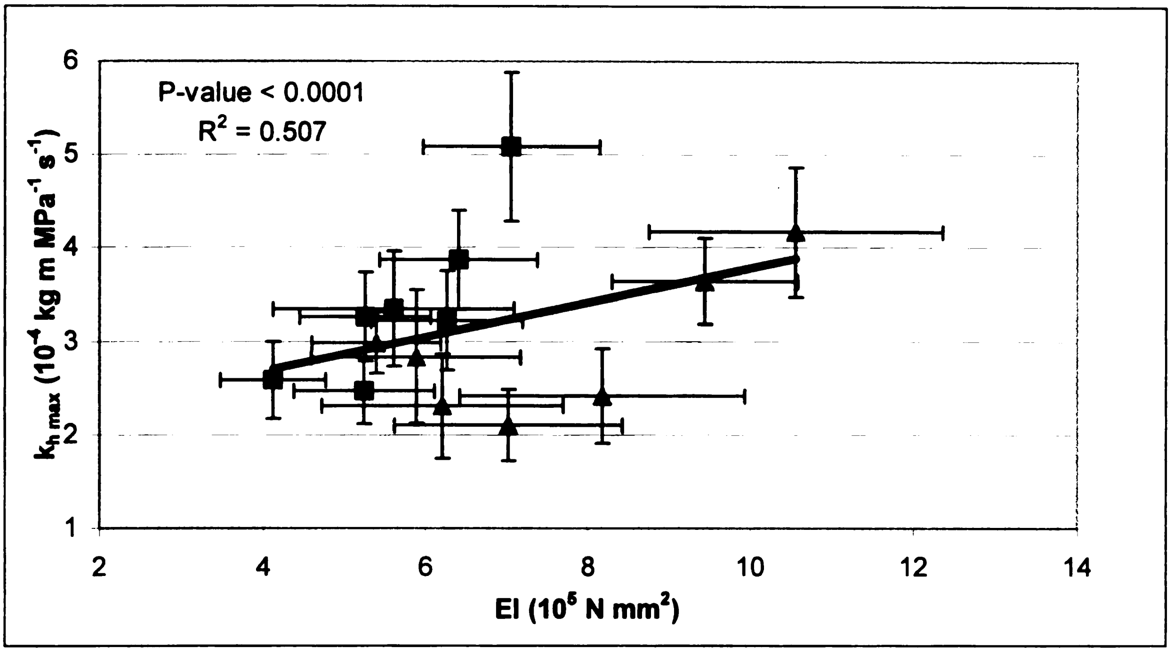

Figure 1. Maximum hydraulic conductivity ($k_{h\,max}$) versus flexural rigidity (EI). Means of each hybrid are shown as control (■) or MP (▲), N=166. Vertical bars are 1 SE of $k_{h\,max}$ and horizontal bars are 1 SE of EI.

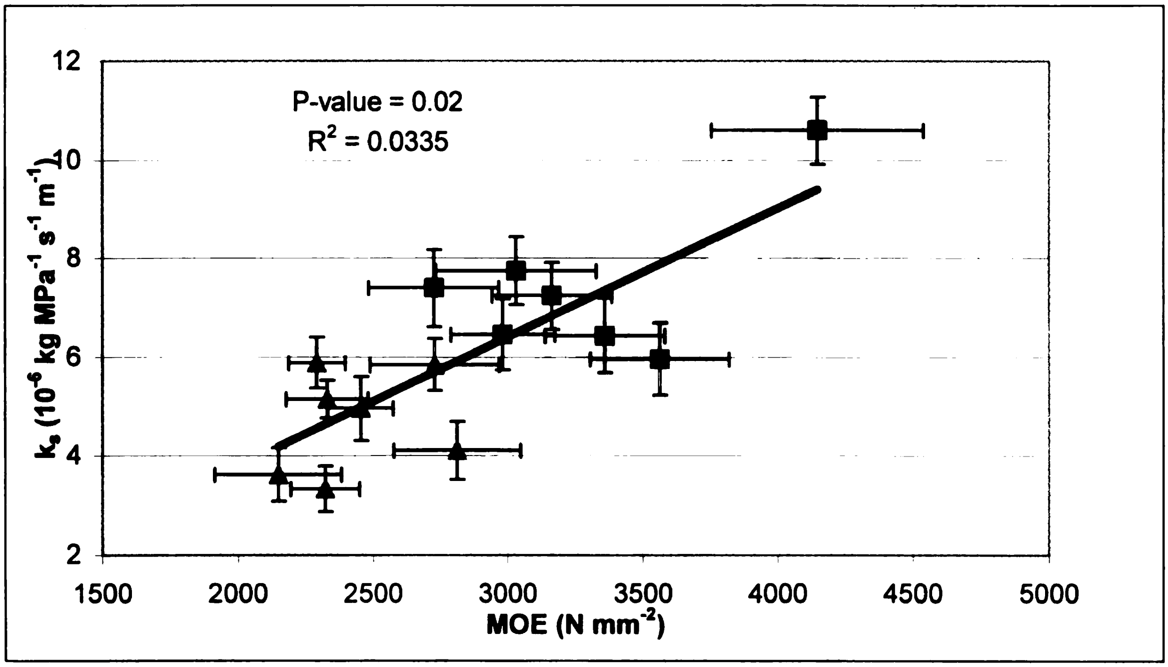

Figure 2. Specific conductivity (k_S) versus modulus of elasticity (MOE). Means of each hybrid are shown as control (■) or MP (▲), N=166. Vertical bars are 1 SE of k_S and horizontal bars are 1 SE of MOE.

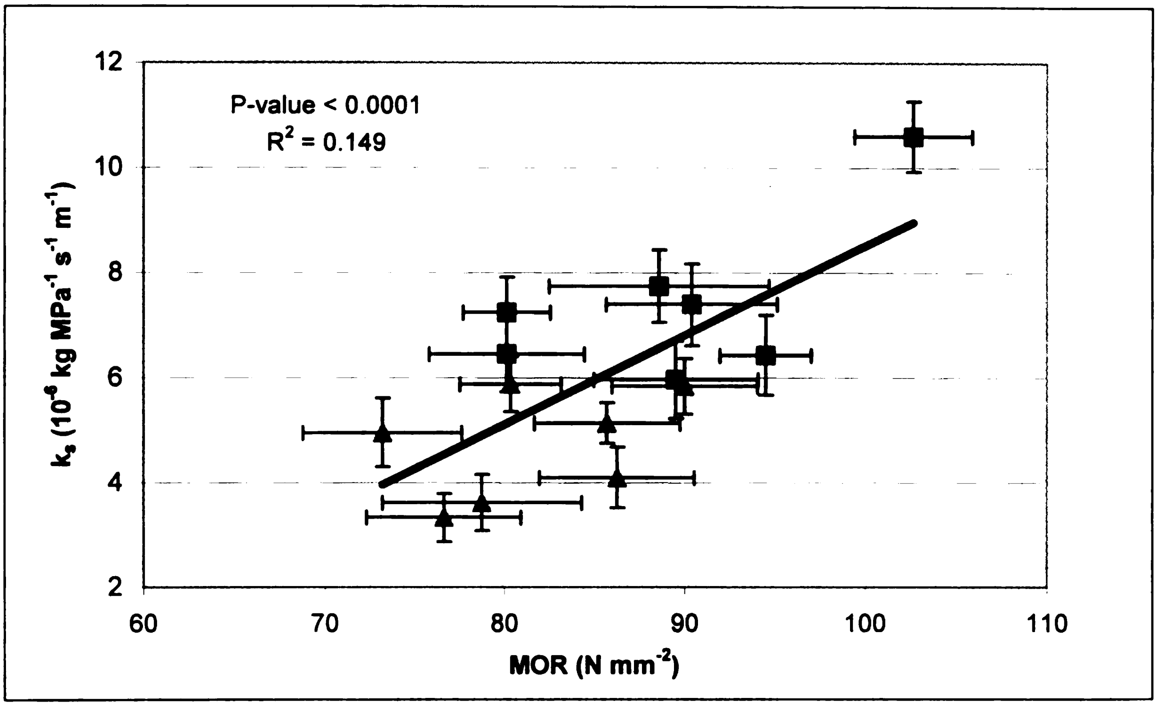

Figure 3. Specific conductivity (k_S) versus modulus of rupture (MOR). Means of each hybrid are shown as control (■) or MP (▲), N=166. Vertical bars are 1 SE of k_S and horizontal bars are 1 SE of MOR.

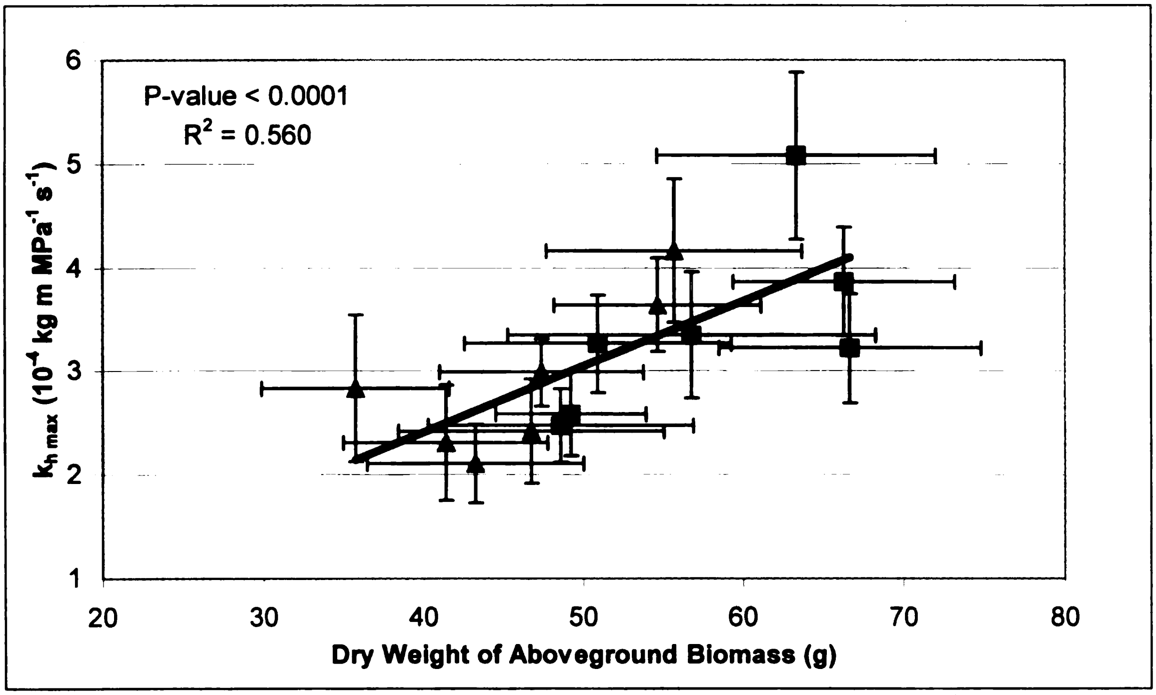

Figure 4. Maximum hydraulic conductivity ($k_{h\ max}$) versus aboveground biomass. Means of each hybrid are shown as control (■) or MP (▲), N=166. Vertical bars are 1 SE of $k_{h\ max}$ and horizontal bars are 1 SE of aboveground biomass.

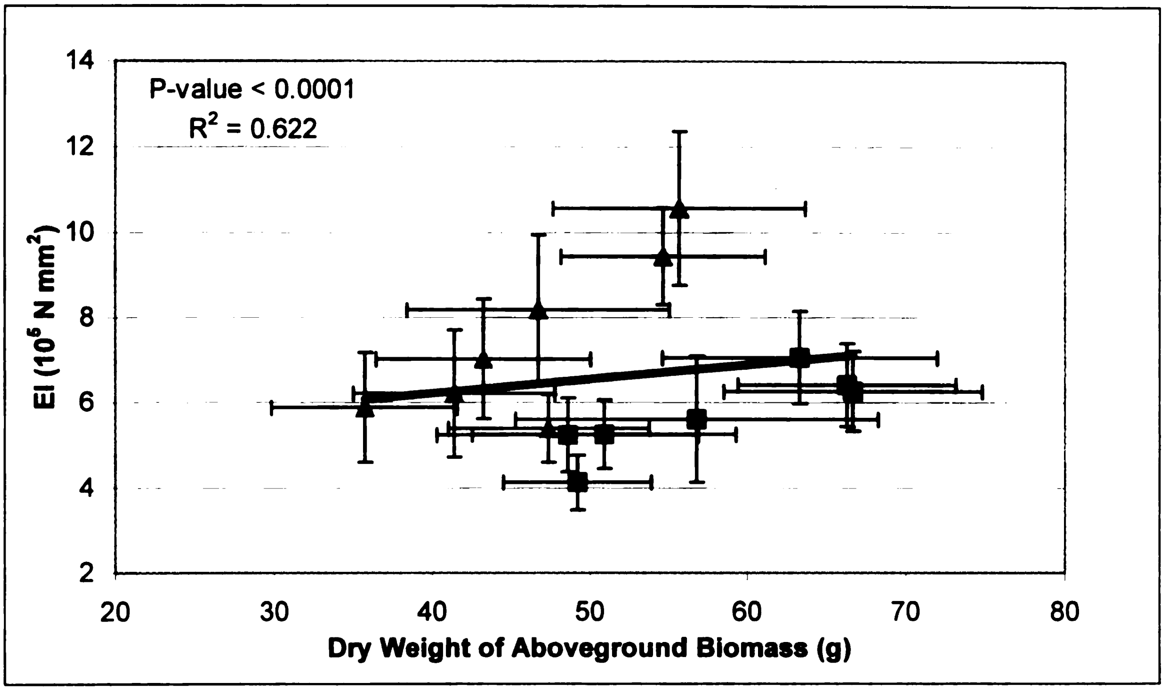

Figure 5. Flexural rigidity (EI) versus aboveground biomass. Means of each hybrid are shown as control (■) or MP (▲), N=166. Vertical bars are 1 SE of EI and horizontal bars are 1 SE of aboveground biomass.

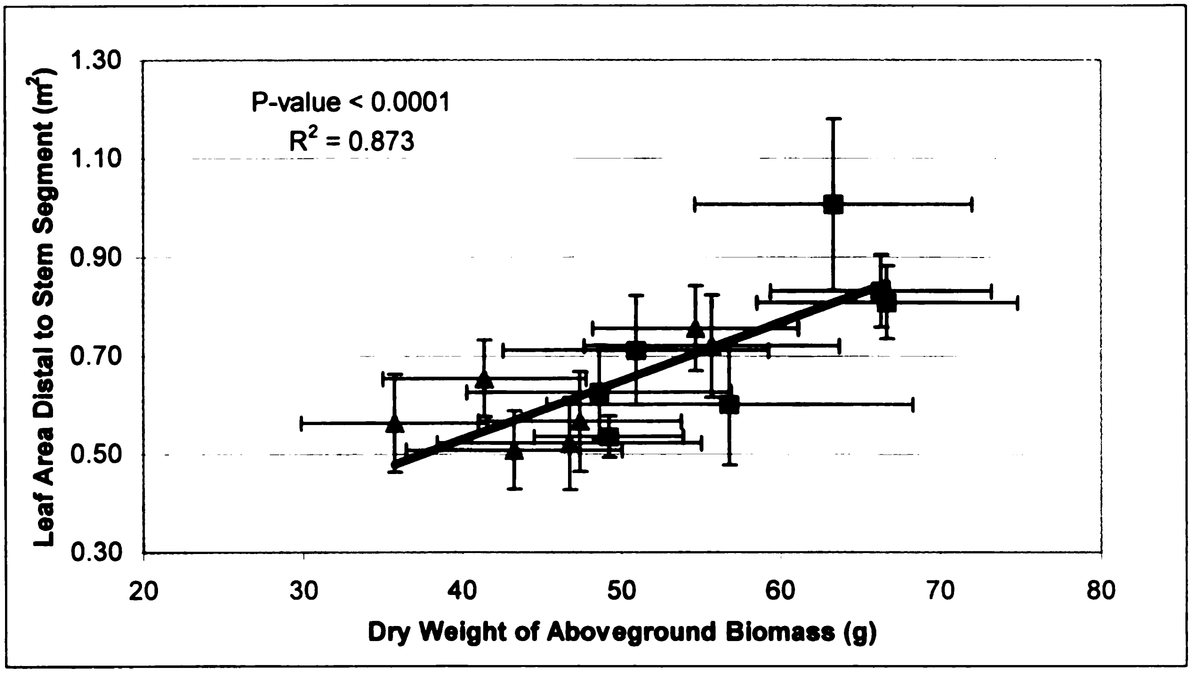

Figure 6. Leaf area versus aboveground biomass. Means of each hybrid are shown as control (■) or MP (▲), N=166. Vertical bars are 1 SE of leaf area and horizontal bars are 1 SE of aboveground biomass.

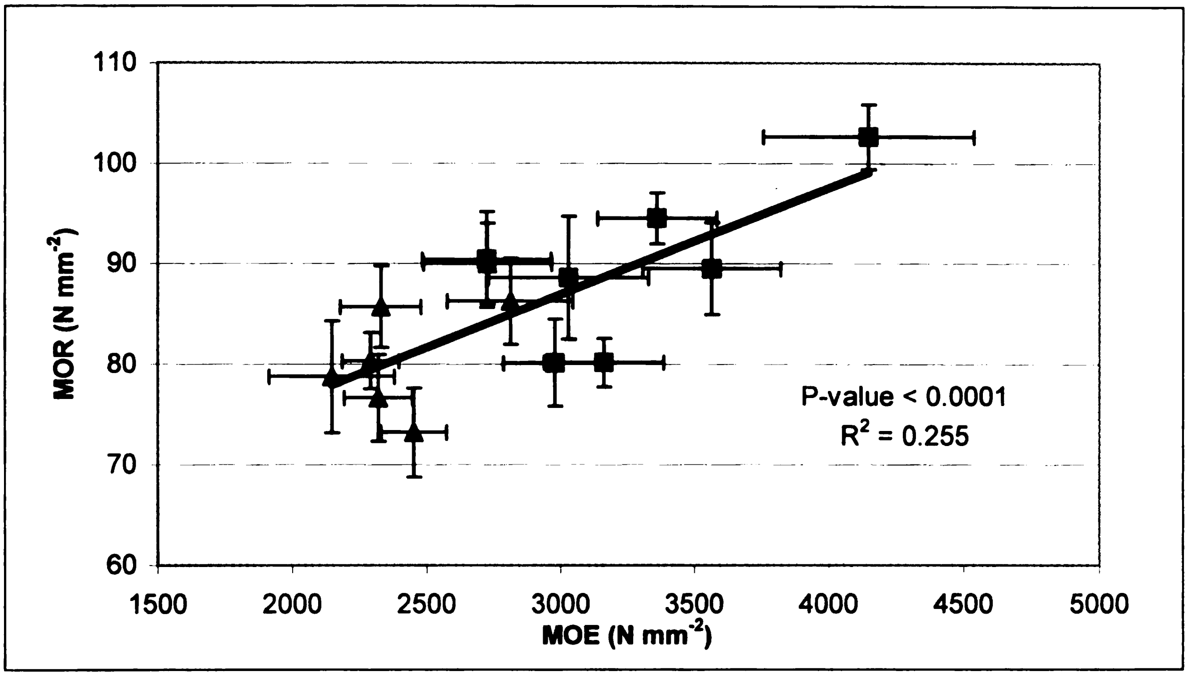

Figure 7. Modulus of rupture (MOR) versus modulus of elasticity (MOE). Means of each hybrid are shown as control (■) or MP (▲), N=166. Vertical bars are 1 SE of MOR and horizontal bars are 1 SE of MOE.

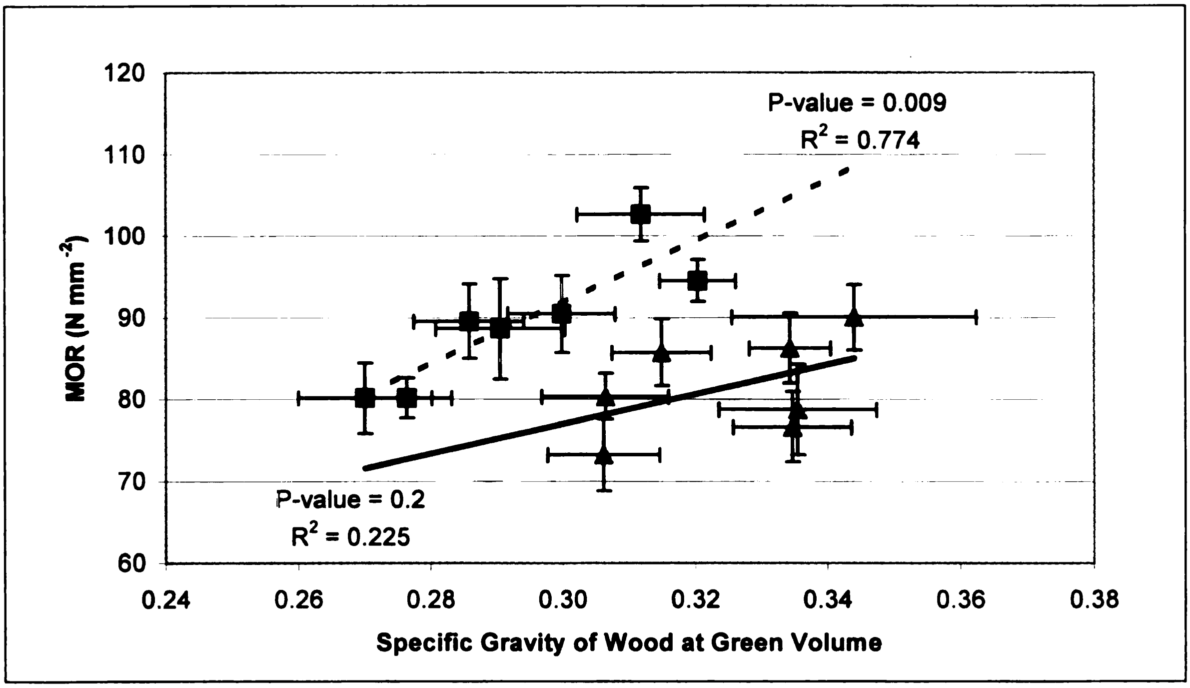

Figure 8. Modulus of rupture (MOR) versus specific gravity of wood. Means of each hybrid are shown as control (■) or MP (▲), N=166. Vertical bars are 1 SE of MOR and horizontal bars are 1 SE of specific gravity of wood.

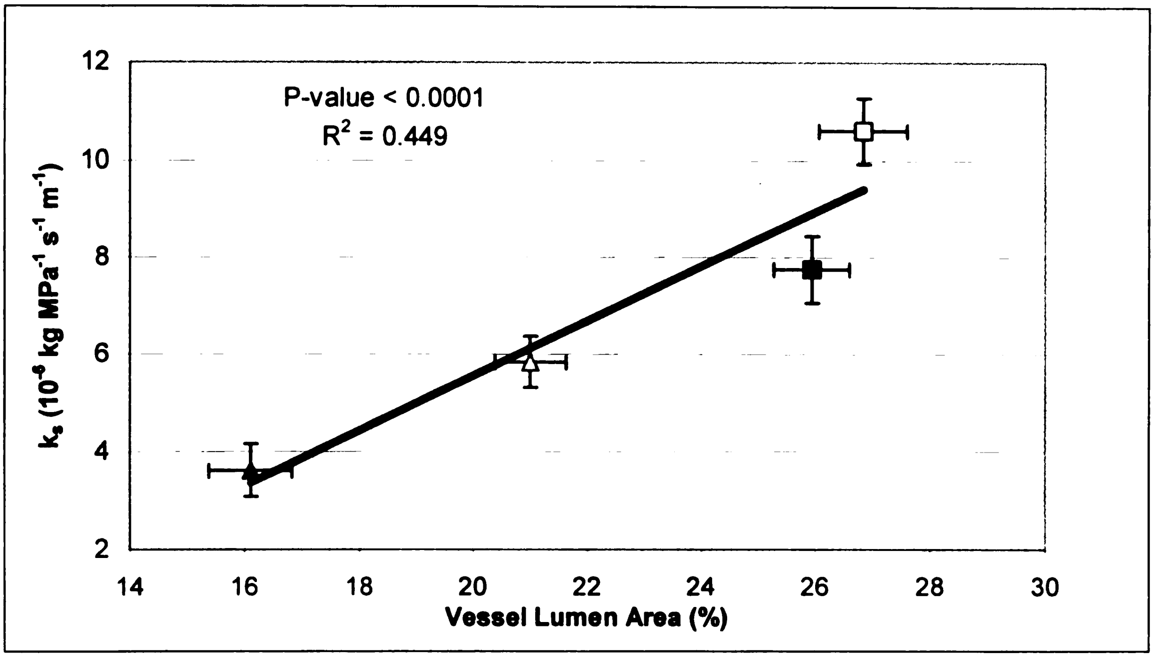

Figure 9. Specific conductivity (k_S) versus vessel lumen area. ■ = Hybrid 46-158 Control; □ = Hybrid 19-61 Control; ▲ = Hybrid 46-158 MP; Δ = Hybrid 19-61 MP; N=32. Vertical bars are 1 SE of k_S and horizontal bars are 1 SE of vessel lumen area.

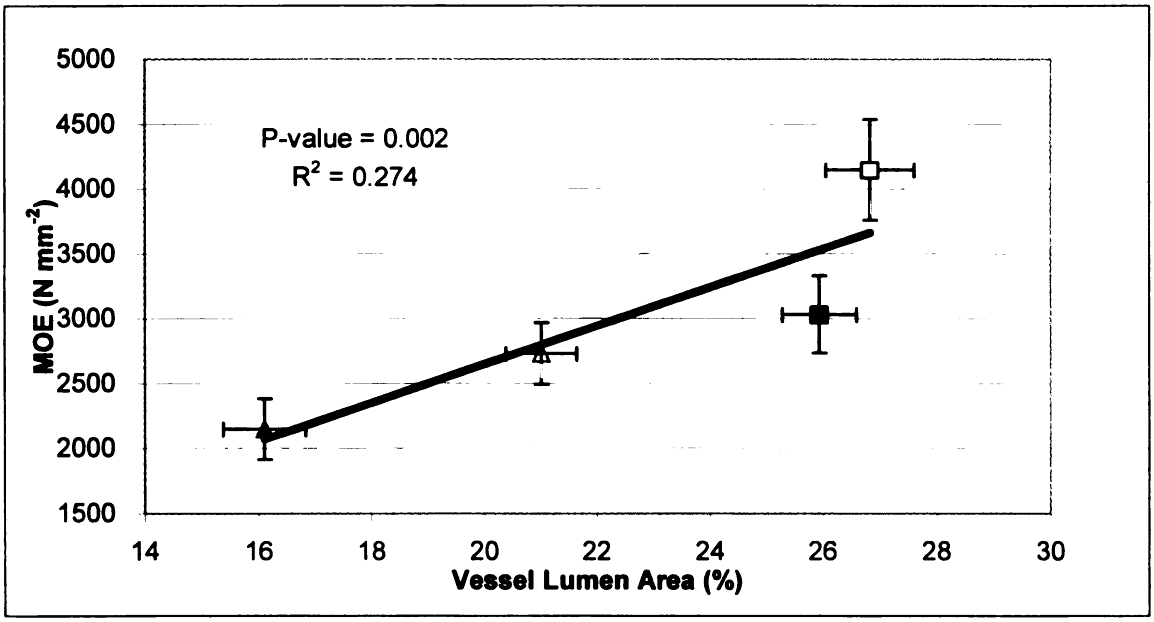

Figure 10. Modulus of elasticity (MOE) versus vessel lumen area. ■ = Hybrid 46-158 Control; □ = Hybrid 19-61 Control; ▲ = Hybrid 46-158 MP; Δ = Hybrid 19-61 MP; N=32. Vertical bars are 1 SE of MOE and horizontal bars are 1 SE of vessel lumen area.

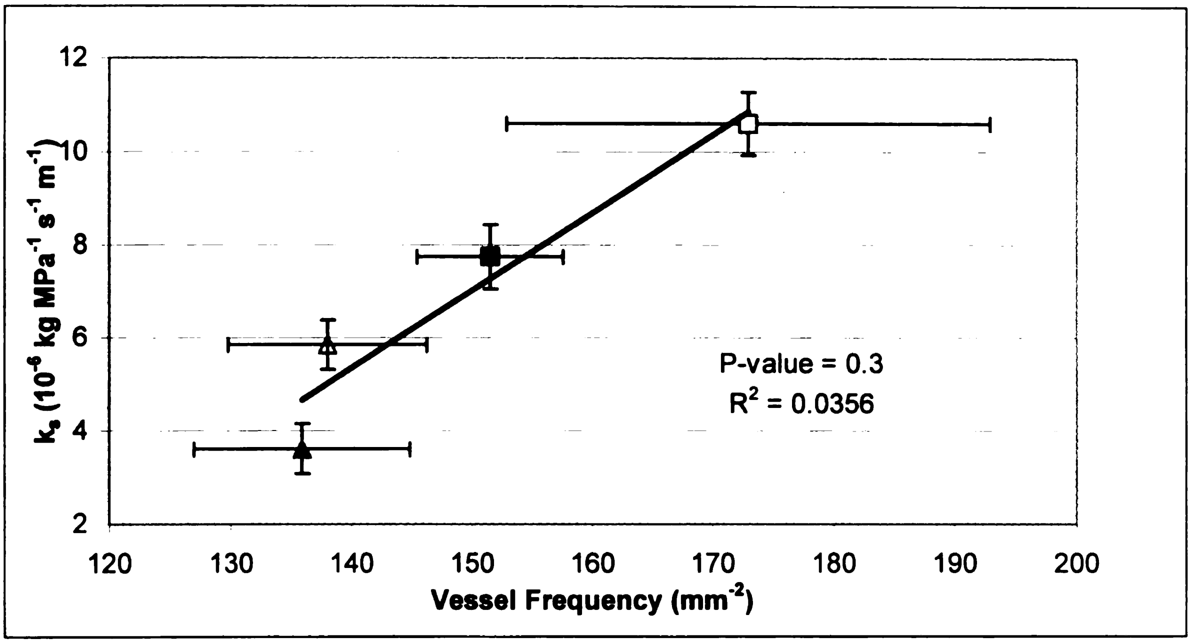

Figure 11. Specific conductivity (k_S) versus vessel frequency. ■ = Hybrid 46-158 Control; □ = Hybrid 19-61 Control; ▲ = Hybrid 46-158 MP; Δ = Hybrid 19-61 MP; N=32. Vertical bars are 1 SE of k_S and horizontal bars are 1 SE of vessel frequency.

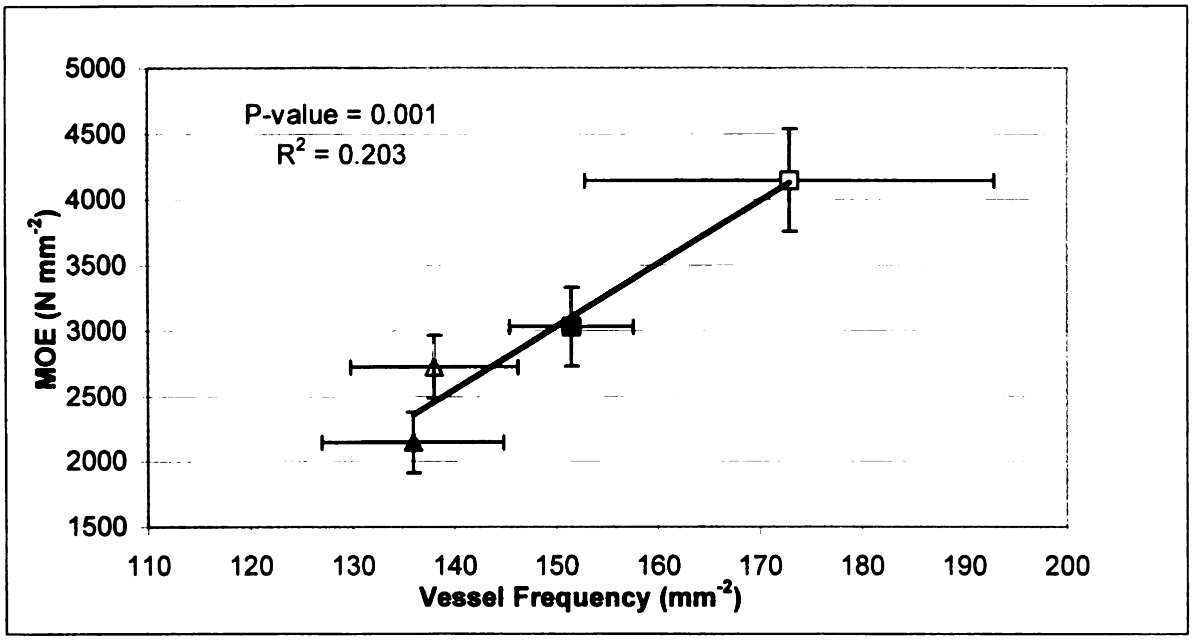

Figure 12. Modulus of elasticity (MOE) versus vessel frequency. ■ = Hybrid 46-158 Control; □ = Hybrid 19-61 Control; ▲ = Hybrid 46-158 MP; Δ = Hybrid 19-61 MP; N=32. Vertical bars are 1 SE of MOE and horizontal bars are 1 SE of vessel frequency.

DISCUSSION

Summary of major findings

The allocation of biomass of these seven hybrid poplars in response to mechanical perturbation (MP) was consistent with results from the literature as detailed in the discussion below: height growth and leaf area decreased, and radial growth increased. Mechanical perturbation of clones increased the mechanical strength on a whole stem basis, another result consistent with the literature. Previously unknown, specific conductivity (k_s) of stems decreased when subjected to MP. Total aboveground biomass also decreased with MP. Despite decreased conductivity and increased mechanical strength when subjected to MP, overall, maximum conductance ($k_{h\ max}$) was positively correlated with flexural rigidity (EI) and percent vessel lumen area, suggesting some hybrids have superior conductivity and mechanical strength. As expected hybrids varied in conductivity and mechanical strength, but not total aboveground biomass, suggesting there is an opportunity to select clones with superior mechanical strength and conductivity.

The mechanical perturbation (MP) treatment

The present results support thigmomorphogenetic theory: radial growth increased, stem elongation decreased and leaf area decreased with MP (Neel and Harris 1971, Jaffe 1976, Telewski and Jaffe 1986a, Telewski 1995). The total effect of MP on tree growth and development resulted in a more compact growth form. Telewski (1995) described how this ultimately reduces the speed-specific drag of the crown and maintains geometric similarity between the stem diameter and height growth, producing a greater margin of safety against mechanical failure under windy conditions.

Radial growth increased with MP, a result consistent with Hybrids 47-174 and 11-11 studied by Pruyn et al. (2000). The effect of MP on stem elongation of Hybrids 47-174 and 11-11 was significant in the present study. Pruyn et al. (2000) found that elongation of stems subjected to MP was decreased in Hybrid 11-11 and increased in Hybrid 47-174; however, neither was significantly different from controls. One explanation for this discrepancy may be that significantly more growth occurred in the 70-90 day MP treatment of this study than Pruyn et al. (2000) found in a 60-day treatment. It is also possible that the time of year the studies were conducted, despite being grown in the greenhouse, influenced the growth rate as well. This study was conducted March through May while the study by Pruyn et al. (2000) was conducted in July and August, the hottest months in a greenhouse in Michigan and the end of the growing season.

Mean leaf area per tree decreased for each hybrid poplar when subjected to MP, a result consistent with Telewski and Jaffe (1986a), Telewski and Pruyn (1998), Cipollini (1999), and Cordero (1999). Net photosynthesis was not measured in this study, however Ferree and Hall (1981) reported a decrease in net photosynthesis and transpiration in response to MP. It is possible MP decreased the net production of photosynthate in these clones in addition to altering biomass allocation. Clearly, total aboveground biomass and leaf area were positively correlated in this study.

External loading forces such as wind must be transferred down the stem to the roots and then into the ground in order to prevent mechanical failure of the tree (Stokes and Mattheck 1996). Examining the effect of MP on tree root development was not addressed in this study, however it may be useful since reduction of wind sway and MP modifies secondary growth in lateral roots (Jacobs 1954, Fayle 1976, Stokes et al. 1995, Stokes et al. 1997). Jacobs (1954) found that sway increased the diameter growth of roots as far as two feet from the base of the trunk of *Pinus radiata* (Monterey pine). Stokes et al. (1995) found that although changes in shoot growth were small, significant differences were found in root growth between wind-stressed and control *Picea sitchensis* (Sitka spruce) and *Larix decidua* (European larch). Wind-stressed trees of both species increased almost 60% in the number of roots important for anchorage on the windward side and in *L. decidua* this also occurred on the leeward side. Stokes et al. (1997) showed that flexing of *P. sitchensis* caused significant increases in: course root mass, course root to fine root ratio, total root to shoot ratio, mean cross-sectional root area along the axis of flexing, and the incidence of coarse branches.

These studies all examined the root growth of gymnosperms and concluded the alteration of biomass to the roots should improve the anchorage of trees subjected to wind movement. Studies that examine the effect of MP or wind on root growth of hybrid poplars are lacking in the literature, however, the structure and function of *Populus* root systems is reviewed in Pregitzer and Friend (1996) and several studies of differences among clones in relative quantity of carbon allocation to root systems are reviewed in Hinckley et al. (1989).

The alteration of biomass due to MP resulted in clones that were shorter and had thicker stems. These physical changes affected the mechanical properties of the stems. Flexural rigidity increased in clones subjected to MP while modulus of elasticity (MOE) and modulus of rupture (MOR) decreased. These results are consistent with Telewski and Jaffe (1986a, 1986b), and Pruyn et al. (2000). Flexural rigidity is the product of MOE and the second moment of cross-sectional area, which scales with stem radius to the fourth power. In this case, the increased radial growth of clones subjected to MP had a greater influence on the calculation of EI than the decrease in MOE which resulted in greater rigidity of the whole stem. Ecologically, the rigidity of the entire stem is important in reducing the susceptibility of the stem to mechanical failure due to wind stress.

Different mechanical results were reported for *Liquidambar styraciflua* (sweet gum) grown under field conditions for 2 years (Holbrook and Putz 1989). Free swaying trees were shorter, increased in diameter, and tended to decrease in MOE. However, whole-tree flexibility (expressed as radians N^{-1}) of free swaying trees was not significantly different from that of constrained trees.

Differences in cell wall composition, the presence of lignin and the angle of the microfibrils, may explain the range of MOE values of the seven hybrid poplars and the relative strength of the wood. The fibers of tension wood typically show the replacement of either the S_2 or S_3 layer by an unlignified layer which in section has a translucent swollen and gelatinous appearance (Wardrop 1964, Panshin and de Zeeuw 1980). This unlignified layer is termed the gelatinous (G) layer and is primarily composed of cellulose.

The presence of gelatinous fibers and lignin content were not examined in the present study, however, Pruyn (1997) found gelatinous fibers and observed lignin in the fiber, vessel, and ray cell walls of control and MP stems of Hybrids 47-174 and 11-11.

Pruyn (1997) also found that total ligninthioglycolic acid (LTGA lignin) decreased in MP stems. When young shoots of *Populus eramericana* cv 'Ghoy' were inclined to induce the formation of tension and opposite wood, the formation of gelatinous fibers on the upper face of the stem was observed (Jourez and Avella-Shaw 2003). Tension wood is the reaction wood formed on the upper sides of branches or leaning stems in dicotyledonous angiosperms and is characterized by the presence of gelatinous fibers, which have lower lignin and higher cellulose content compared to normal fibers (Telewski et al. 1996). In a study of tension wood in *Magnolia obovata* and *M. kobus* which are considered to be primitive angiosperms, the amounts of lignin decreased in the cell walls of fiber tracheids, especially with great decrease in proportion of guaiacyl units in lignins (Yoshizawa et al. 2000). Pruyn (1997) concluded that flexure wood shared some characteristics of tension wood since lignin content also decreased with MP. Mattheck and Kubler (1995) proposed that the decrease in lignin typical of tension wood serves to increase the ability of stems to yield under bending stress. In the present study, decreased lignin content may be another reason for the observed decrease in MOE of stems subjected to MP.

The second explanation for difference in relative strength of cell walls is the angle of the microfibrils. The behavior of wood cells is based to large degree on the structure of their secondary walls, specifically the helical arrangement of the microfibrils in the S_2 layer, which provides a mechanism for absorbing energy and preventing structural failures (Dickison 2000). Microfibril angle and density accounted for approximately 95 percent of the variation in wood stiffness (longitudinal MOE) of longitudinal sections of *Eucalyptus delegatensis* (Evans and Ilic 2001).

Telewski (1989) found that microfibril angle increased in tracheids of *Abies fraseri* (Fraser fir) that differentiated under the influence of flexure. Lindström et al. (2002) found that MOE negatively correlated with microfibril angle of young *P. radiata* clones. In other words, as the microfibril angle increased the elastic properties of the stem decreased. In the present study, a change in microfibril angle in stems subjected to MP may explain the increase in whole stem rigidity. A change in microfibril angle could also affect the strength of the cell walls in directions not measured using the Universal Instron Machine for 4-point bending.

Wood with a specific gravity of 0.36 or less, like the T x D hybrid crosses used in this study is considered light (Panshin and de Zeeuw 1980). Wood specific gravity at green volume of *Populus trichocarpa* was reported as 0.31 with *P. deltoides* at 0.37 (Forest Products Laboratory 1999). The hybrids used in this study have wood specific gravities that fall near *P. trichocarpa*. Mechanical perturbation increased the wood specific gravity of these poplar clones, a result consistent with tension wood of *P. deltoides* (Lassen 1959) and free swaying *L. styraciflua* (Holbrook and Putz 1989). Wood formed under the influence of MP was denser but had lower lignin content according to the results of Pruyn (1997). It is surprising there was no correlation between specific gravity and MOE in the present study especially since there is a linear relationship between MOE and wood density, the amount of cell wall material in a given volume of wood (Lindström et al. 2002). This discrepancy may be due to the juvenility of the wood of the poplar clones used in this study.

Conductivity of these hybrids was previously unknown. Mechanical perturbation decreased stem conductance ($k_{h\ initial}$ and $k_{h\ max}$) and specific conductivity (k_s, conductivity per conductive xylem area). Specific conductivity decreased 10-45% depending on the hybrid when clones were subjected to MP. This result was expected since the flexure wood of angiosperms shares many characteristics with tension wood. Tension wood is characterized by the presence of fewer and smaller vessels than normal wood and a corresponding increase in the proportion of fibers (Wardrop 1964). In other words, conductivity decreases because the frequency of vessels decreases and the vessels are smaller when stems are subjected to MP. In fact this was observed in the MP stems of Hybrids 46-158 and 19-61. Reduction of k_s corresponded to decreases in percent vessel lumen area, hydraulic diameter, vessel frequency, and average and maximum vessel lumen diameter.

Vessel frequency of Hybrids 46-158 and 19-61 was greater than the reported values of mature *P. deltoides* and *P. trichocarpa* (26-100 mm^{-2}, Panshin and de Zeeuw 1980), however average vessel diameter of Hybrids 46-158 and 19-61 was lower than reported values of either parent (51-150 μm, Panshin and de Zeeuw 1980). The range of values for vessel data reported here for Hybrids 46-158 and 19-61 are within the range found for Hybrids 47-174 and 11-11 by Pruyn (1997).

As is the case in the present study, Pruyn (1997) also showed that vessel frequency, percent vessel lumen area, and average vessel diameter decreased in stems subjected to MP. These results are generally consistent with Telewski and Jaffe (1986a, 1986b) as well as studies of dicotyledonous angiosperms. Neel and Harris (1971) observed a reduction in vessel element length and diameter in flexed *L. styraciflua* as compared with the vessel elements of untreated trees. Jourez et al. (2001) found vessel frequency and porosity were significantly lower in tension wood than in opposite wood of young inclined stems of *Populus euramericana* cv 'Ghoy'. Jourez et al. (2001) also observed that vessel elements in tension wood were 2.5% longer than in opposite wood, a result inconsistent with flexed *L. styraciflua* (Neel and Harris 1971). Mechanical perturbation decreased tracheid length in *A. fraseri* (Telewski and Jaffe 1986a) and *P. taeda* (Telewski and Jaffe 1986b). Data on vessel element length was not collected in the present study and it remains unknown whether the vessel elements of these hybrid poplars increased or decreased in length due to MP. The average vessel element length for mature *P. trichocarpa* is reported as 58 ± 9 μm (Panshin and de Zeeuw 1980). More than 90% of *Populus* vessels are shorter than 15 cm in length (Blake et al. 1996). Investigation of ray anatomy of flexure wood is lacking in the literature, however rays of tension wood in *P. deltoides* decreased in size (Kaeiser and Boyce 1965) and were more numerous in *Populus euramericana* cv 'Ghoy' (Jourez et al. 2001).

Positive correlations between mechanical and hydraulic properties

The present study suggests there is an opportunity to select clones with superior mechanical strength and conductivity because there was a significant positive correlation between $k_{h\,max}$ and EI (whole tree basis) and between k_s and MOE (tissue area basis). This correlation was not expected and contradicts the results of other research (Wagner et al. 1998, Spicer and Gartner 2002, Woodrum et al. 2003). Several explanations are possible for the contradiction: Wagner et al. (1998) used different stem segments to test conductivity and mechanical strength of chaparral shrubs; Spicer and Gartner (2002) used *Pseudotsuga menziesii* (Douglas-fir), a gymnosperm having one cell type to function both in water transport and support; and Woodrum et al. (2003) used lateral branches of five

Acer species (likely to have tension wood present) which differ anatomically compared to the wood of hybrid poplars.

There was a significant positive correlation between k_s and percent vessel lumen area for these hybrids but we did not collect data on fiber lumen area. Jourez et al. (2001) found a negative correlation between the proportion of vessel lumina and the proportion of fiber lumina including the G-layer. This is interesting because in the present study, stems subjected to MP had decreased percent vessel lumen area but increased specific gravity. This would mean as wood formed when subjected to MP, fewer vessels were produced and they occupied less of the stem cross-sectional area. As a result, more fibers must be present. The presence of more fibers supports the observed increase of flexural rigidity of clones subjected to MP. In the future, analysis of fibers may confirm this assumption for these hybrid poplars.

Although fibers were not examined in the present study, three fiber variables are considered to be important in determining the physical characteristics of pulp and paper: fiber density, fiber length, and fiber strength (Panshin and de Zeeuw 1980). Fiber length is particularly important since it determines to a large extent the physical and mechanical properties of paper (Koubaa et al. 1998). The average diameter of libriform fibers of mature *P. deltoides* and *P. trichocarpa* ranges from 26 to more than 30 µm (Panshin and de Zeeuw 1980). Fiber length of mature *P. trichocarpa* was reported as 1.38 ± 0.19 mm (Panshin and de Zeeuw 1980). Koubaa et al. (1998) showed that fiber length varied considerably by clone type and height in the tree for ten different *Populus* x *euramericana* clones. Tension wood fiber length has been described as longer, and sometimes of equivalent length or shorter (Jourez et al. 2001). Examination of the effect of MP on fiber properties may be of interest especially in hybrid poplar clones harvested specifically for pulp.

The positive correlation between $k_{h\ max}$ and EI is strong in the case of these seven hybrid poplars. The relationship remained significant even when Hybrid 19-61, the triploid that exhibited great conductance and mechanical strength, was removed from regression analysis. Triploids have a phenotype that is biased toward the parent from which it carries two genomes (Bradshaw and Stettler 1993). Clearly, Hybrid 19-61 exhibited greater growth compared to the six diploids. In addition, triploids typically have longer and wider fibers (Stettler et al. 1996), anatomical characteristics that offer an explanation for the observed greater mechanical strength compared to the diploids.

The relationship between $k_{h\ max}$ and EI is also supported by evidence that both k_s and MOE were positively correlated with percent vessel lumen area. Clearly, some of the clones have both great mechanical strength and water conduction. When subjected to MP, Hybrid 19-61 had the most radial growth compared to the six other hybrids (diploids) as well as higher vessel frequency in stem cross-section compared to Hybrid 46-158. In other words, when stems of Hybrid 19-61 were subjected to MP, more wood developed in the stem and a greater proportion of the stem was in the form of vessels. Both of these phenomena may explain the superior conductivity and mechanical strength of Hybrid 19-61.

Due to the heterosis exhibited by some of these clones in terms of conductivity and mechanical strength, it may be advantageous to select poplar clones based on mechanical strength (MOE, MOR) and conductivity (k_s). Testing conductivity and mechanical strength of older stems is needed however. The stems tested in this study were juvenile and it remains unknown whether the wood properties of these seven hybrid poplars are similar to seven-year-old stems harvested for woody biomass production.

Silviculture of hybrid poplar in plantations is intensive and rotations are short (five to eight years for fiber alone and up to 15 years for solid wood products); therefore, a large portion of the wood produced will be in the juvenile core (DeBell et al. 2002). The wood formed each year gradually assumes the character of mature wood and the progression from juvenile to mature wood differs among wood properties and species (Panshin and de Zeeuw 1980, Zobel and Sprague 1998). For *P. deltoides*, the juvenile/mature wood demarcation point for mechanical properties and specific gravity is about 17 to 18 years, with cell length and fibril angle stabilizing about a year or two later (Bendtsen and Senft 1986). Therefore, hybrid poplars in plantation are harvested well before maturity.

Juvenile wood is generally weak, lower in density, and composed of cells which are shorter in length and vessels narrower in diameter when compared to those found in mature wood (Telewski et al. 1996, Zobel and Sprague 1998). Large microfibril angles, short fibers with thin walls, and low percentages of latewood in the annual ring all contribute to low strength and stiffness in juvenile wood (Bendtsen and Senft 1986). However differences between juvenile core and mature wood of diffuse-porous hardwoods such as *Populus* are often quite small, so that sometimes product quality in operational forestry is not affected (Zobel and Sprague 1998). In fact, DeBell et al. (2002) showed that average wood density of Hybrids 11-11 and 47-174 was similar for the first three years (0.37 g cm^{-3}), decreased to 0.335 g cm^{-3} at age four and five, and finally increased to 0.40 g cm^{-3} at age nine. On the other hand, mean fiber length of the two clones consistently increased each year through age nine, starting at approximately 0.55 mm and ending at approximately 0.95 mm (DeBell et al. 2002).

In addition to the concerns about differences between properties of juvenile and mature wood, selection of poplar clones with great mechanical strength and water conduction should be accompanied by other considerations. For example strong shoots with superior water conduction may have another liability not yet identified (e.g. vulnerability to xylem embolism or attack by insects and pathogens). Field tests of older individuals are needed to determine the economic potential of specific clones.

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