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THIGMOMORPHOGENESIS:
RESPONSES OF TWO POPULUS HYBRIDS TO MECHANICAL STRESS

By

Michele Lynn Pruyn

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ABSTRACT

THIGMOMORPHOGENESIS: RESPONSES OF TWO POPULUS HYBRIDS TO MECHANICAL STRESS

By

Michele L. Pruyn

Two poplar hybrids from the cross *Populus trichocarpa* x *P. deltoides*, 47-174 and H11-11, showed developmental acclimation in response to mechanical stress. Increased radial growth from the control was evident in mechanically perturbed stems of both hybrids, which resulted in their increased flexural stiffness EI. The increase in EI in the stressed stems was due to increased I, second moment of cross-sectional area, which overcame decreases in Young's Modulus, E. This decrease in E occurred despite the observed decrease in vessel lumen area per mm^2. Lower values in E suggest an alteration in the material composition of the stem, facilitating it's ability to absorb wind-induced, bending energy. Flexed stems of both hybrids also showed differences from the control in lignin content and composition.

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CHAPTER ONE: General Responses of Woody Stems to Gravity and Wind/Flexure Stress and An Introduction to the Genus *Populus*

As relatively stationary organisms, trees are subject to countless physical challenges from their immediate environment. Some of these stresses are perhaps more obvious than others. The sight of a fallen tree might bring to mind causes such as old age, a severe storm, pathogen attack, or environmental pollutants. The identification of wind as the culprit for such an event is a less obvious conclusion. By nature it is elusive; wind is evident only as an afterthought, recognized as the fluttering of leaves, or the moan of twisting branches. Wind's deceptive ability to travel invisibly makes it no less dangerous than a bolt of lightening or a deadly fungus to an unsuspecting tree. As it turns out, however, trees are not unprepared for the impact of wind's unpredictable gusts, or its steady blows.

Introduction

The impact of flexure, or bending on a tree's growth and development is immense. Wind, which is the primary agent responsible for flexure, is prevalent in almost any land environment. The degree of flexure to which a tree might be subjected varies according to its specific geography, and its stand density and position within a forest (Grace 1977, 1980). As freestanding, stationary organisms, trees must develop a structural design that will provide endurance to environmental stresses that range from mild to extreme. This design must also serve to be functionally efficient in the tree's photosynthesis and water-conductance (Givnish 1995, Gartner 1995). When considering the effects of wind-induced stress upon tree stems, it is important to analyze changes that occur morphologically, mechanically, anatomically, and biochemically. Thigmomorphogenesis is the name given to the associated physiological and morphological responses of plants to wind and other mechanical stresses (Jaffe 1973).

Characteristics of the wood and fibers in coniferous species can be altered by wind indirectly when a stem is displaced from its vertical alignment with gravity's vector, resulting in the formation of compression wood (Telewski 1995). A direct effect of wind's influence in a coniferous stem is the formation of flexure wood (Telewski and Jaffe 1986a,b, Telewski 1989, Telewski 1995). Likewise, wind can indirectly cause the formation of tension wood in dicot angiosperm species when the stem is displaced from its vertical orientation. The direct effect of wind flexure on dicot angiosperms is currently unknown. The occurrence and character of flexure wood formation needs to be described in these species. Flexure wood formation has been positively identified as a direct response to wind, or motion, and is independent of a gravitropic response (Telewski 1989, 1995). The direct and indirect alterations that occur in wood and fiber quality will be discussed for coniferous and woody dicotyledenous angiosperm species.

Effects of Gravity on Wood Formation

Wood that is produced on the longer radius of eccentric stems and branches is referred to as reaction wood. It usually occurs in stems that have been displaced from their vertical alignment with respect to the force of gravity for a critical period of time (the gravitropic presentation time). The gravitropic presentation time is the length of time necessary to initiate the formation of reaction wood. Wind can therefore indirectly initiate reaction wood formation in tree stems. Trees that are swayed by wind in one direction for the required presentation time have received a secondary wind stress and will show the formation of reaction wood in an otherwise vertical stem (Telewski 1989, 1995). Reaction wood is both anatomically and chemically different from the wood in stems that have not been displaced for the required gravitropic presentation time (Creber 1975, Panshin and DeZeeuw 1980, Robards 1969, Timell 1986 a,b,c).

Angiosperms and gymnosperms differ in the location and composition of reaction wood. Metzger (1893) coined the terms *tension wood* and *compression wood* to describe the two different types of reaction wood associated with softwoods and hardwoods. Reaction wood in angiosperms is known as tension wood. It develops on the upper sides of branches, or displaced (leaning) trunks, which gives the branch or stem an elliptical shape. Tension wood has been found in the roots of many angiosperms; but they are usually not elliptical (Patel 1964).

In gymnosperms and primitive angiosperms lacking vessels, reaction wood is known as compression wood, and it forms on the lower sides of branches and leaning trunks (Creber 1975). The occurrence of compression wood in roots is much less consistent than in stems and branches, and is thus an extremely controversial subject. Certain species of conifers have been reported to have no compression wood in their roots, whereas others are said to have compression wood only in surface-roots that are exposed to light

(Westing 1965, 1968). Timell (1986b) provides a review of the literature on root compression wood.

Reaction wood formation is not exclusive to branches and leaning stems. Mature trunks of *Pawlonia* and *Catalpa*, for example, showed evidence of tension wood in various regions around the perimeter of the stem (Patel 1964). Another interesting example is the high occurrence of tension wood in stems of some angiosperms, especially *Populus,* which have not been displaced from their vertical alignment with respect to gravity. This altered wood formation has been associated with the rapid growth and intensive management practices (that is, cultivation, irrigation, and fertilization) of short-rotation forestry (Parham et al. 1977, Isebrands and Bensend 1972, Isebrands and Parham 1974).

Reaction wood formation in tree stems can be described using the terminology of stress and strain relationships from mechanics (Levitt 1980 a,b). The external factor acting upon the organism is known as the stress, whereas the strain is the organism's subsequent response, or deformation (physical, or chemical change). Strains are considered plastic if irreversible physical or chemical changes have occurred in the organism. When a tree has been permanently displaced from its vertical orientation with respect to gravity's vector, it has undergone a plastic strain. Reaction wood formation is considered to be a recovery attempt by the tree. It is a mechanism to realign the stem to a vertical orientation, or to maintain and stabilize the position of a branch or a leaning trunk when it is at an angle with respect to the vector of gravity (Panshin and DeZeeuw 1980). Despite this function of reaction wood to return leaning trees to the vertical position, the entire stem does not attain the original vertical position (Telewski 1995). The trunk usually maintains a 'sweep' in the stem, whereas the crown is usually reoriented to the vertical position. Elastic strains (discussed in the next section) are stress-related responses that are reversible physical or chemical changes. An example is a tree that sways in response to wind, but returns to its original vertical position before the gravitropic presentation time has elapsed. If this temporary displacement from the vertical does not exceed the presentation time, an elastic strain upon a tree stem will not result in reaction wood formation (Telewski 1995).

The occurrence of compression wood, as well as its anatomical features and chemical composition, has been characterized extensively by the work of Creber (1975), Panshin and DeZeeuw (1980), Robards (1969), and Timell (1986 a,b,c). The pith is eccentric and the growth rings are elliptically shaped. This morphology contrasts the centered pith and the nearly circular rings of stems that have remained vertical with respect to gravity's vector. Compression wood is also tinged with a red color, hence the early common name 'redwood' (Robards 1969, Timell 1986 a,b,c). On a microscopic level, the cross-sectioned tracheids are rounded, unlike normal tracheids that are angular-shaped. As a consequence of this atypical, rounded shape, large intracellular spaces occur where three or four tracheids meet. Compression wood tracheids are also up to 25% shorter than normal (Robards 1969). Cell walls of compression wood tracheids and fibers are approximately twice as thick as normal and are heavily lignified. The cellulose polymer content is correspondingly reduced, while galactose is increased (Panshin and DeZeeuw 1980). Usually the innermost secondary wall layer (S_3) is lost, and the S_2 layer is characterized by a greater helical angle (45°) than in normal wood, which is usually between 15° and 20° (Panshin and DeZeeuw 1980). This increased spiral angle is extremely evident in the cell wall due to the presence of helical checks that follow the microfibrillar angle (Panshin and DeZeeuw 1980, Robards 1979). These checks are actually a system of radial cavities that form during the development of the S_2 layer as a result of the deposition of large amounts of lignin (Boyd 1973, Coté et al. 1968).

When considering the combined effects of the alterations that occur in the reaction wood of coniferous species, the significance of Metzger's (1893) choice of the term *compression wood* becomes clear. As the pressure builds in the thickening cell wall of developing compression wood tracheids, lateral expansion in the cell is prevented because the cellulose microfibrils are arranged quasi-transversely to the cell axis. This constraint is non-existent in the longitudinal direction of the cells. The effect of compression wood is thus explained by its property to expand longitudinally (Mattheck and Kubler 1995). It is this compressive force on the lower sides of branches, or leaning stems that either stabilizes, or realigns them with respect to gravity's vector.

Tension wood in angiosperms has also been well characterized. Within the xylem of tension wood, the vessels are similar to those in normal wood. The vessels are smaller in diameter and less numerous (Panshin and DeZeeuw 1980). The most notable changes in cell character are evident in the fibers. With the decrease in the number of vessels in tension wood, there is an increase in fibers. The fibers are usually narrower and longer than those in normal wood. Tension wood fibers also have thickened cell walls which is a characteristic of the walls of compression wood fibers. Unlike compression wood fibers, this increase in cell wall thickness is not due to heavy lignification, but is usually a result of the presence of gelatinous layer within the wall. The gelatinous layer (G-layer) is highly characteristic of the fibers in tension wood. Fibers with a gelatinous layer are especially prevalent at the beginning of a growth ring; normal fibers often resume at the end of each ring (Robards 1969). Splitting between fiber cells is a frequent occurrence within the gelatinous layer, especially under compressive forces (Robards 1969). Under the tangential tensile and compressive loading of wind stress, for example, the rays tend to shear instead of bend, which causes them to lose their interlocking properties (Mattheck and Kubler 1995). Shear cracks then develop and run perpendicularly to the wind load.

The G-layer is poorly lignified, or unlignified, and its cellulose microfibrils are oriented nearly parallel (about 5°) to the long axis of the cell. This special layer always occurs on the lumen side of the cell wall, and frequently appears swollen and buckled (Panshin and DeZeeuw 1980). The G-layer in tension wood fibers will occur in addition to, or actually replace the S_3 and/or S_2 layers (Panshin and DeZeeuw 1980, Robards 1969). The total lignin content in tension wood is reduced from that in normal wood only if represented as a percentage of the total wood (Creber 1975, Panshin and DeZeeuw 1980). However, when considering the individual secondary cell wall layers of tension wood fibers, the lignin concentration will vary. While lignin is largely absent from the G-layer in tension wood, it exists in the S_1 and S_2 layers of tension wood fibers in the same amount as in normal fibers. Since the S_1 and S_2 layers in tension wood fibers are thinner than those in normal wood, the concentration of lignin in these layers is actually higher than in the same layers of normal wood (Timell 1969). With the overall reduction in the amount of lignin in tension wood fibers, there is a corresponding increase in cellulose, which is concentrated in the G-layer (Robards 1969).

As is the case with *compression wood* in gymnosperms, Metzger's (1893) choice of *tension wood* describes perfectly the mechanism of reaction wood in angiosperms. The consideration of the anatomy of tension wood brings to the light the stark contrast of its mechanism to that of compression wood. Tension wood contracts in the axial direction, thus pulling a leaning stem into a vertical position, or maintaining the position of a branch (Mattheck and Kubler 1995). Because the cellulose microfibrils are aligned parallel to the cell wall axis, they act to contract the tension wood cells longitudinally. No such restraint exists in the lateral direction, which allows the cell to expand transversely. The swollen, cellulose-rich gelatinous layer meanwhile maintains the shape of the tension wood cell. This parallel orientation of the cellulose microfibrils imparts a longitudinal component of shrinkage to the tension wood, which functions by magnifying an internal tensional force (Panshin and DeZeeuw 1980, Telewski et al. 1996). It is this tensile force on the upper sides of branches, or leaning stems that either stabilizes, or realigns them with respect to gravity's vector.

Effects of Mechanical Perturbation, or Wind on Wood Formation

Wood that is produced in response to temporary flexure from wind-sway is referred to as flexure wood (Telewski 1989). Reaction wood formation is not initiated because the tree's displacement from its original vertical position is not permanent and does not exceed the gravitropic presentation time. Dynamic wind loading imposes alternating compressive and tensional forces within the tree stem (Telewski 1995). The tree is returned to its original position by its stem tissues, which are resisting the wind's force.

This resistance within the stem of the tree does not return the stem immediately to its previous orientation; instead, an oscillation is created, as the stem swings past its original position (Holbo et al. 1980, Mayer 1987, Gardiner 1995). Eventually, if no further wind stimulation is initiated, the swaying motion will decay until the tree has returned to its original position. This type of action by wind upon trees is considered a primary wind stress. The temporary bending of tree stems in response to primary wind stress is considered an elastic strain, which subsequently induces the formation of flexure wood (Telewski 1989, 1995). The formation of flexure wood is therefore considered a direct response to wind stress. This chain of events strongly impacts a tree's growth and development.

Flexure stress promotes a shortened growth form and greater stem taper in developing trees (Telewski 1995). More specifically, a wind-stressed tree will usually show a reduction in stem elongation and/or an increase in radial growth. The increase in radial growth observed in flexure-stressed trees is asymmetrical, with the long axis in the direction of flexure (Telewski and Jaffe 1986b, Telewski and Pruyn 1997). These findings have been reported in angiosperm as well as gymnosperm species (Jacobs 1954, Jaffe 1973 1980, Grace 1977, Hoblrook and Putz 1989, Neel and Harris

1971, Telewski and Jaffe 1981 1986a, Telewski and Pruyn 1997). A wind-stressed tree may also have shorter branches and smaller leaves. This overall design of the tree has the basic effect of enhancing the tree's ability to remain vertical under greater stresses, while simultaneously diminishing strain by reducing speed-specific drag of the crown (Telewski and Jaffe 1986a).

The influence of wind loading on tree roots has recently received attention in gymnosperm species (Stokes et al. 1997). Rigidity was reported to increase in the leeward roots of *Pinus pinaster*, and maturation strains were positive, possibly indicating the formation of an abnormal wood type (Stokes et al. 1997). A complete study of the wood properties and formation of roots in response to wind stress has not yet been carried out in either angiosperms, nor gymnosperms.

In conifers flexure wood has been characterized extensively in the context of its similarity to compression wood and to wood developed in the absence of flexure (normal wood) (Telewski 1989). Since all trees in the natural environment are subject to frequent bending by wind, it is somewhat misleading to denote wood that has developed in the absence of flexure as normal. Strictly for purposes of comparison, however, wood produced under controlled-environmental conditions without any mechanical perturbation will be considered in this chapter as *normal.* Anatomically, the flexure wood of coniferous species has tracheids that are shorter and narrower than normal (Telewski 1986a, 1989). Flexure wood tracheids approach those typical of compression wood (Telewski 1986a, 1989). Unlike compression wood tracheids, however, those of flexure wood are not rounded with large intercellular spaces, and the S_2 cell wall layer is not thickened (Telewski 1989, Telewski and Jaffe 1986b). Also, the flexed stems of *Pinus taeda* seedlings did not show a significant increase in lignification when compared to the normal stems (Telewski and Jaffe 1981). When considering the cellulose microfibrils in the same species, however, Telewski (1989) found the angles to approach the higher angles found in compression wood.

Anatomical characteristics of the flexure wood of angiosperm species are not as extensively documented as in conifers. Neel and Harris (1971) found a reduction in the length of fibers of mechanically perturbed stems of *Liquidambar styraciflua.* Also, vessel members of the shaken trees showed a decrease in both diameter and length as compared to the control. Currently, evidence of other changes in the anatomy of flexure wood is lacking from the literature.

Grain angle and growth ring density are both affected by mechanical loading stress. Quirk et al. (1975) found that mechanical stress caused a change in grain angle in *Pinus radiata*. The angle was increased from a 5° angle (from the vertical) to one of 17.5°. Telewski and Jaffe (1986a) found an increased grain angle in the mature trees growing on the windward side of Roan Mountain as compared to those growing on the leeward side. This increased grain angle results in a slightly tighter grain helix for wind-stressed trees. Telewski and Jaffe (1986a) also recorded such responses for flexure-stressed trees grown under laboratory conditions. Average growth ring density increased in the flexed stems of *Abies fraseri*, both as grams per cubic centimeter and as the ratio of cell wall to cell lumen area, when compared to the control stems (Telewski 1989). Telewski (1990) also reported increased density (g cm^{-3}) and radial growth for flexed *Pinus taeda*.

Considering the combined effects of these two responses to wind-stress brings to light how trees are acclimating to the load of wind. The increased growth ring density provides the stem with a greater mass and volume of xylem per square centimeter of cambial surface (Telewski 1995). The tightened grain helix, which tends towards a spiral grain in the stem, diminishes the compressional load of wind in the stem. The compressive stresses act perpendicularly to the spiral orientation of the tracheids, which reduces the risk of splitting and the shear in between the tracheids (Mattheck and Kubler 1995). The tensional load of wind acts in the direction of the helical orientation of the tracheids, thus enhancing the tree's ability to remain vertical while reducing strain (Telewski and Jaffe 1986a).

The morphological and anatomical changes that occur in mechanically stressed trees have an effect on the biomechanical properties of the woody stem. The wind/flexure-directed increase in radial growth of a stem results in an increase in the second moment of cross-sectional area (I) when compared to control stems. Young's elastic modulus (E), however, was reported to decrease in the flexed stems of *Abies fraseri* and *Pinus taeda* (Telewski and Jaffe 1986a, b, Telewski 1990). The net effect is an increase in flexural stiffness (EI) in the flexed tree stem, which is the product of the elastic modulus and the second moment of area. The increased radial growth has a greater impact on the calculation of EI, than does the reduced elastic modulus. This tendency is explained by the formula for I, which scales with the stem radius to the fourth power (Wainwright et al. 1976). Holbrook and Putz (1989) recorded that whole-tree flexibility (radians/N) was strongly influenced by shading and staking the angiosperm, *Liquidambar styraciflua*.

Shaded, staked plants were significantly more flexible (decreased in stiffness) than the unshaded, free-swaying control or staked plants; there was no significant difference between the whole tree flexibilities of the latter two treatments. They attribute the observed difference to variation in stem allometery, however, and not to experiment-induced changes in material (wood) properties. Wood density (g cm^{-3}), and wood flexibility did not differ significantly among the treatments.

Summary

Stability of architecture is of great importance to trees. Their ability to achieve lofty heights and immense volumes in growth demands that they develop a structure that will be mechanically sound under various environmental stresses. It is well known that plants orient their growth with respect to the force of gravity (Cholodny 1927, Went 1937). In the case of trees, this orientation becomes increasingly crucial with the development of expansive branching patterns about the stem axis. Introducing agents of environmental stress, such as wind, complicates the situation even more. The impact of the combined forces of flexure and gravity upon tree growth, development, and design is difficult to understand. Isolating a specific developmental response and attributing it to either flexure or gravity is nearly impossible to do in the natural environment. Under controlled conditions, the influences of these environmental stresses have been separated into gravitropic and thigmomorphogentic responses, for gravity and wind/flexure, respectively.

The responses of tree stems to these environmental stresses can be viewed from the perspective of design principles for biological structural systems (Wainwright et al. 1976). Stems withstanding a compressive load develop accordingly; that is, compression elements are short and have enlarged perimeters. Compression wood, which is the response of gymnosperm and some angiosperm stems to displacement from their alignment with respect to the force of gravity, has the characteristics of compression elements. Stems under a tensile load develop tensile elements, which involves the parallel arrangement of high modulus wood fibers (Wainwright et al. 1976). These fibers are relatively inextensible under tensile stresses exerted in the direction of the fibers, yet are readily pliable under compressive stresses.

Tension wood, which is the response of angiosperm stems to displacement from their alignment with respect to gravity's vector, has the characteristics of tensile elements. Under windy conditions, trees experience a multitude of loading patterns, such as tension, compression, torque, shear, and bending. Following the logic of design principles, a natural hypothesis would be that flexure wood must share some structural similarities with reaction wood (compression wood in gymnosperms and tension wood in dicot angiosperms).

The tendency of flexure wood in gymnosperm species to have compression wood elements has been extensively documented (Quirk et al. 1975; Telewski and Jaffe 1986a, b; Telewski 1989). Characteristics of the flexure wood in angiosperm species are currently undefined. Whether flexure wood in angiosperms acquires tensile or compressive elements depends on which type of stress is more prevalent in windy conditions, tension or compression. Only tensile forces are resisted by purely tensile elements; however, tension and shear are resisted by compressive elements (Wainwright et al. 1976). Determining whether flexure wood in angiosperms has more characteristics of tension wood, or of compression wood will answer the question of whether it is compressive, or tensile elements in a tree stem that are vital to maintaining its vertical position under windy conditions. This knowledge will also answer the question of whether angiosperm wood is capable of developing compressive elements, or if its physiology dictates the formation of tensile elements exclusively.

Description of the Study Genus, *Populus*

Populus belongs to the family Salicaceae along with *Salix* and some other genera (depending on whether segregates of either of these two genera are recognized). Species of the genus *Populus* spread clonally, via root-borne sucker shoots (soboliferous) (Eckenwalder 1996). They are all deciduous (or semievergreen) and single-trunked. Often they play a role, ecologically as vegetative pioneers because they are fast-growing. This type of growth is facilitated by the ability of their shoots to continue growing after bud burst (Critchfield 1960). Throughout the growing season they are initiating, expanding, and maturing leaves (neoformed or late leaves).

Poplars are wind-pollinated and have flowers that are borne in pendent racemes (catkinns, aments). The arrangements of the flowers are usually similar for the males and females of the same species. The seeds have a coma of cottony hairs on parietal placentas in thin-walled capsules (Eckenwalder 1996).

The wood of the genus *Populus* is diffuse-porous, which means there is no distinct difference between the size of the latewood and earlywood vessels (Panshin and DeZeeuw 1980). Tree age in *Populus* spp. is important in determining the width and basal area of both sapwood and heartwood (Telewski et al. 1996). In *P. tremuloides,* for example, heartwood begins to form after five years of growth. A combination of vessel elements, libriform wood fibers, and axial and ray parenchyma make up the xylem (Telewski et al. 1996). Willows (*Salix* spp.), who share the same family as *Populus*, have wood that is very similar to *Populus*, based on gross anatomical features. One characteristic difference, however, occurs in the structure of the wood rays. *Salix* spp. rays are essentially heterocellular, whereas *Populus* spp. rays are homocellular (Panshin and DeZeeuw 1980). Within the genus *Populus*, it is nearly impossible to differentiate species considering only anatomical features. Panshin and DeZeeuw (1980) were able to use vessel density and vessel element length to separate various species.

The radial and axial systems of parenchyma cells within *Populus* woody tissue have a variety of physiological functions (Telewski et al. 1996). These systems are functionally and physically connected, where the radial system is composed of uniseriate, 'homogenous' ray parenchyma, and the axial system parenchyma cells are arranged vertically within the wood tissue. Some of the noted functions of these cells include the storage and distribution of food materials (Kramer and Kozlowski 1979), the exchange of solutes with the water and conducting elements (Sauter 1980, 1981a,b), the excretion and secretion of products, or the compartmentalization and sealing of wounds (Shigo 1984). Sauter and van Cleve (1989, 1990) and Sauter (1972) have characterized the various functions of the ray parenchyma cells within the uniseriate rays of *Populus*. These rays can be divided into the categories of contact cells, isolation cells, and cells of the contact cell rows. The assignment of a particular ray cell to a given category is based upon the cell's position within the ray and the physiological function in regards to storage product mobilization and deposition (Telewski et al. 1996).

The study species for the experiments within this thesis are two hybrids from the cross of *P. trichocarpa* and *P. deltoides*. *Populus* *trichocarpa* (black cottonwood) has been the focus of a 19 year program for evaluation of short-rotation forestry's effects on biomass production. There has been an emphasis, within the program, for genetic improvement via selective breeding and the creation of hybrids. The Pacific Northwest has currently allocated 7,000 ha to hybrid poplar plantations, and the area is designated to be tripled before the end of this century (Stettler et al. 1993). Harrington and DeBell (1996) have identified potentially wind-tolerant and -intolerant hybrids. The wind-tolerant hybrid for the study is 47-174, and the intolerant is 11-11. Cuttings of these hybrids were obtained and successfully rooted in a nursery at Michigan State University.

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CHAPTER TWO: Morphological and Biomechanical Responses of Two *Populus* Hybrids to Mechanical Stress

Introduction

Jaffe (1973, 1980) created the term Thigmomorphogenesis to describe the physiological and morphological responses of plants to wind and other mechanical stress. Trees grow in response to their specific environment, consequently acclimating growth processes that are both physiological and biochemical. The tree can prevent stem failure from the prevalent wind loading conditions by reducing drag and/or increasing mechanical strength (Telewski, 1995; Telewski and Jaffe, 1986a, b).

Stress and strain terminology from mechanics has been applied to plant physiology by Levitt (1980a). Stress is the external force acting upon an organism, and the resulting response, or deformation in the organism is the strain. Strains are further classified by their degree of elasticity, or plasticity. An elastic strain occurs when a tree sways in response to wind and subsequently, returns to its original, vertical orientation. If a tree undergoes irreversible physical or chemical changes and is permanently displaced, the induced strain on the tree is plastic.

The physical manifestation of these wind-induced strain responses in various plant species is of great interest to many researchers and crop specialists. A forest stand in which the trees experience plastic strains can have a tremendous impact on its productivity, or survivability. Trees that do not buckle under the forces of wind may recover from permanent displacement from their vertical orientation by forming reaction wood. Reaction wood as a part of the gravitropic response in trees has been described extensively by Timell (1986a, b, c). Trees respond to elastic strains as well. Telewski (1995) provided an extensive summary and review of the recent developments in the research of the various responses of trees to wind stress. In terms of economic value, the occurrence of these altered formations in response to stress could potentially have detrimental effects on the quality of wood produced.

When attempting to understand how trees react to wind-stress, many researchers have adapted mechanical models to explain the various developmental responses that occur (Levitt 1980a, b). Wilson and Archer (1979) used the constant surface strain hypothesis to describe the biomechanical relationship between height and stem diameter of a tree. Supporting this hypothesis, Mattheck proposed a mechanism in trees for maintaining a balanced structural design, in which the assorted loading forces are distributed to the surface of all load-carrying parts of the tree (Mattheck 1990, 1991). It is interesting to note that experiments which have been performed using these models suggest that trees are "over-designed" as free-standing columns, with buckling limits that greatly exceed the theoretical buckling limit determined by McMahon (1973) (Gartner, 1991; Mencuccini et al., 1997).

The focus of this experiment is to describe the morphological and biomechanical responses of *Populus* hybrids to mechanical perturbation (MP). These responses have been characterized extensively in gymnosperms by the work of Telewski and Jaffe (1986a, b) and Telewski (1989, 1990). Their findings indicate an increase in radial growth and a decrease in stem elongation in response to MP. They also reported an increase in the flexural stiffness (EI) and a decrease in Young's Modulus (E) in the mechanically perturbed stems. Such experiments have been conducted to a lesser extent in dicotyledonous angiosperms. Neel and Harris (1971) reported anatomical changes in *Liquidambar styraciflua* in response to MP. Holbrook and Putz (1989) noted changes in the mechanical properties and wood density of *Liquidambar styraciflua* when exposed to wind sway. Recently, Telewski and Pruyn (1997) discussed the effects of incremental levels of flexure stress on stem elongation and radial growth in *Ulmus americana* L. seedlings. The influence of mechanical flexure remains otherwise undefined in dicot angiosperms.

For this investigation, two hybrids were selected based on characteristics of wind-firmness described by Harrington and DeBell (1996). A cross between *Populus deltoides* and *Populus trichocarpa* resulted in hybrids that exhibited differences with regard to wind tolerance. The hybrid, 11-11, was identified as wind intolerant and more susceptible to wind toppling and wind throw. Among the various hybrids in the study, 11-11 had the highest amount of above-ground biomass per unit of root area, and the highest ratio of above-ground biomass to cross-sectional area of the mean root (Harrington and DeBell 1996). 11-11 had the highest value for stem slenderness when expressed as height divided by root-collar diameter. It also had the lowest rate of stem taper from groundline. The study also identified a wind tolerant hybrid, 47-174. In comparison with the other hybrids from the study, the wind-firm hybrid had the highest rates of lower stem taper, the lowest amounts of above-ground biomass per unit of cross-sectional root area, and the largest roots (Harrington and DeBell, 1996).

The present study reports the developmental acclimations of the hybrids, 47-174 and 11-11, to mechanical flexure. The hypothesis tested was that the hybrid-specific traits of *Populus* would dictate how they respond morphologically and mechanically to mechanical stress. These responses to mechanical stress will then be discussed in terms of their biological and ecological significance.

Materials and methods

Culture of the plants

The trees used in this study were two hybrids from the F_1s of a cross between *Populus trichcarpa* and *Populus deltoides*. One hybrid, 47-174, is designated a wind tolerant hybrid and the other, 11-11, is considered an intolerant variety, based on the field studies of Harrington and DeBell (1996). Stem cuttings of these plants have been successfully rooted during the past two years in a nursery at MSU. The rooted cuttings were transplanted into 7.3 x 7.3 x 22.8cm Utility Band pots (Anderson Die and Manufacturing Company, 2425 S.E. Moores St., Portland, OR, U.S.A.). The growth medium used for these plants was a combination (in a ratio of 1:1) of a high porosity professional planting mix, supplied by Baccto (Michigan Peat Company, P.O. Box 980129, Houston, TX 77098) and a recycled and sterilized sandy mix, supplied by the MSU greenhouse. After sprouting was initiated, the cuttings were transplanted twice with growth. The first transplant was to an 8L pot and the final, to an 18L pot. These cuttings were cultivated in a greenhouse under photoperiods ranging from 12 to 14 hours. Diurnal temperatures were maintained at 25°C. The original stem cuttings were left to grow for two weeks undisturbed after the final transplanting. During this time, new stem shoots were initiated for each cutting. At the end of two weeks, one leader stem was selected from every cutting for uniform height and stem width. Any other shoot growth from the main stem was removed. Each selected leader stem was then staked.

Eighteen individuals of each hybrid were used. There were two treatments: control and mechanical perturbation (20 flexures). Nine of the 47-174 hybrids were control, and the other nine were flexed mechanically in the NE to SW direction. The same design was used for the 11-11 hybrid. The flexure treatments were administered once daily for the months of July and August of 1996. Before treatments began, a point of flexure was chosen for the stems. The flexure point is the place on the stem that is grasped each time for treatment with the thumb and forefinger. The other hand is used to shake the stem by grasping the plant at several centimeters above the flexure point. This flexure point was designated as the point just above the fourth node from the base of each leader stem. The daily treatment consisted of gently grasping the stem at the flexure point and bending it back and forth, not exceeding more than 45° from the original vertical position of the stem. After treatment, the stem was returned to the vertical position and restaked.

Over the course of the experiment, pesticides were applied three times. Azatin and Talstar were used at a rate of 2-4mL per 4 Liters of water. This chemical spray was applied to the subject population twice within two weeks time for the purpose of removing aphids. Avid was applied once in spray form (rate of 1mL per 4 Liters of water) for white fly control.

The seedlings were fertilized using Peters 20-20-20 (Scots-Sierra Horticultural Products Co., 14111 Scotslawn Rd., Marysville, OH 43401) once after one month of treatment, using a Miracle-Gro Siphonex Brass Siphon Mixer (Scotts Miracle-Gro Products, Inc., Port Washington, NY 11050, U.S.A.) to the greenhouse garden hose for application. The concentration applied to the poplars was 465ppm, or 30 grams per 4 Liters of water.

Morphological measurements

The experiment was terminated at the end of two months time. Heights (in cm) of the leader stems were recorded, as well as stem diameter (in mm) at the point of flexure. Diameter measurements were taken in the direction of flexure (NE to SW) and in the direction perpendicular to flexure using a digimatic caliper.

Mechanical properties

After the morphological measurements were recorded, each stem was cut to a length of 14cm. The point of flexure was situated in the approximate center of the 14cm stem lengths. Just before Instron testing, maximum and minimum stem diameters were measured (in mm) with a digimatic caliper at three points along the stem lengths. An average of these diameter measurements was used for the second moment of cross-sectional area, equation (2).

The modulus of elasticity (Young's modulus - E, Nmm^{-2}), the second moment of cross-sectional area (I, mm^4), and the flexural stiffness (EI, Nmm^2) were determined for each specimen using the four-point bending test described in Wainwright et al. (1979). The plastic properties of the stems were measured at room temperature (~20°C) by deflecting the stems using an Instron, model 1331 - retrofitted with 8500 electronics in Michigan State University's Biomechanics Department.

Each stem was bent on a tangential plane with simple beams supporting each end of the stem. The distance between the two supported ends is referred to as the span length (L) (Figure 1). A load of 45.6kg was applied at two points along the 70.0mm span length at a rate of .5mm/sec. The distance between one supported end and the nearest loading point (P) is known as 'a', and was equal to 20.0mm on each end of the stem (Figure 1). The slopes (P/V) of the curve Load vs. Distance Deflected were generated using Microsoft Excel, version 5.1. The slope of each curve, L, and 'a' were inserted into the equation:

$$EI = P/V\,(a^2/12)(3L-4a), \qquad (1)$$

As described in Gere and Timoshenko (1984) to calculate Young's modulus. Inserting P/V into equation (1) generated EI (Nmm^2). E (Nmm^{-2}) was calculated by dividing the EI by I (mm^4). I was calculated using the equation:

$$I = \pi r^4/4, \qquad (2)$$

As described in Gere and Timoshenko (1984).

Data for all parameters are presented as a mean and standard deviation for each treatment. The populations were tested for significant differences within treatment groups using ANOVA in SAS - version 6.11. A multiple comparison among the means of each treatment group (the Duncan's Multiple Range Test) was used to determine which treatments were significantly different.

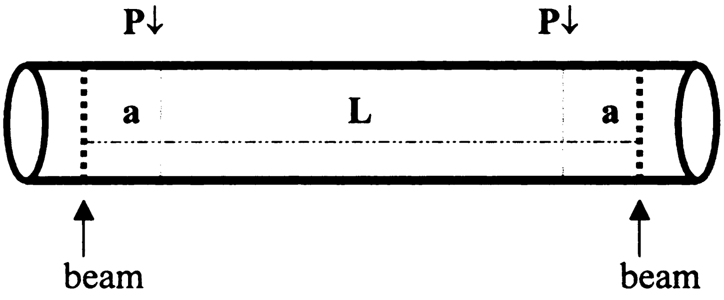

Figure 1: **Instron Set-up:** This diagram represents a 14cm stem segment loaded onto the instron for a four-point bending test. P is the loading force, which was equal to 45.6kg at a rate of 0.5mm/sec. L is the span length, which was 70mm, and 'a', the distance between the loading force and the beam, was 20mm. Adapted from Gere and Timoshenko (1984)

Results

morphological properties

Both control and MP stems of the wind tolerant hybrid, 47-174, were significantly taller than both treatments of the intolerant 11-11s. There was no significant height differences between the mechanically perturbed and the non-perturbed, control stems for either hybrid (Table 1).

By the end of the two-month treatment period, the mechanically perturbed stems showed a significant amount of radial growth over that of the non-perturbed, control stems in both hybrids (Table 2). There was no significant difference between the radial growth in the control stems of the two hybrids (Table 2). Ratios of the stem diameter parallel to the mechanically perturbed (MP) axis and the stem diameter perpendicular to the MP axis were calculated to determine stem roundness. If the calculation approached a value of 1.00, then the stem was considered round. These ratios were calculated using diameters measured pre-treatment, and using the change in diameters after two months of treatment. Pre-treatment ratios showed no significant differences among treatments; that is, each ratio approached 1.00 and was therefore considered round (Table 3). Post-treatment ratios indicate that the mechanically perturbed

Table 1: **Change in Height** (cm) of *Populus trichocarpa* x *P. deltoides* hybrids after two month's exposure to flexure treatments of 20X per day. Each mean (± SD) followed by a different letter is significantly different at P<0.05.

Hybrid - Treatment (n=9)	**Average Height (cm)**
47-174 - Non-perturbed (Control)	94.6±13.0 A
47-174 - Mechanical Perturbation (MP)	104.4±8.5 A
11-11 - Non-perturbed (Control)	83.3±11.5 B
11-11 - Mechanical Perturbation (MP)	77.6±6.7 B

Table 2: **Radial Growth** (mm) of *Populus trichocarpa* x *P. deltoides* hybrids after two month's exposure to flexure treatments of 20X per day. For each measurement, a mean (± SD) followed by a different letter is significantly different at P<0.05. X is the axis parallel to MP, and Y is the axis perpendicular to MP.

Hybrid - Treatment (n=9)	**Radial Growth – X (mm)**	**Radial Growth - Y (mm)**
47-174 - Control	3.25±0.62 C	3.35±0.47 B
47-174 - MP	4.75±0.39 A	4.52±0.41 A
11-11 - Control	3.32±0.65 C	3.35±0.66 B
11-11 - MP	4.18±0.30 B	4.03±0.30 A

stems approached an elliptical shape at the flexure point. The long axis of the ellipse was in the direction of mechanical perturbation. This trend is especially apparent in the 47-174 hybrid, where the mechanically perturbed stems showed a ratio of 1.05; and the controls, a significantly different ratio of 0.97 (Table 3). The 11-11 mechanically perturbed stems also tended toward an elliptical shape at the flexure point, showing a ratio of 1.04. The control stems showed a ratio of 0.99, which was not found to be significantly different from the MP stems (Table 3).

The height to diameter ratio (H:D) was calculated for each stem pre- and post-treatment. Post-treatment calculations included the change in height to stem diameter in the calculation. Prior to the two months of treatment, there were no significant differences in H:D among the trees for 47-174, or 11-11. After treatment, a significant decrease was seen in the MP stems for both hybrids.

Table 3: **The Change in Height to Stem Diameter Ratio and the Radial Growth, X to Y axes, Ratio** (H:D - cm/mm, and X:Y - mm/mm)of *Populus trichocarpa* x *P. deltoides* hybrids after two month's exposure to flexure treatments of 20X per day. X is the axis parallel to MP, and Y (X:Y) is the axis perpendicular to MP. The initial H:D and X:Y ratios of pre-treatment are also shown. In each column, means followed by a different letter are significantly different at $P<0.05$.

Hybrid – Treatment (n=9)	**H:D Mean (pre-treatment)**	**ΔH:D Mean (after 2mos. Treatment)**	**X:Y Mean (pre-treatment)**	**ΔX:Y Mean (after 2mos. treatment)**
47-174 Control	12.56 A	29.67 A	1.03 A	0.97 B
47-174 MP	12.60 A	22.07 C	1.00 A	1.05 A
11-11 Control	9.93 B	25.53 B	1.01 A	0.99 AB
11-11 MP	10.38 B	18.60 D	1.00 A	1.04 A

Table 4: **Mechanical Properties** of *Populus trichocarpa* x *P. deltoides* hybrids after two month's exposure to flexure treatments of 20X per day. For each measurement, a mean (±SD) followed by a different letter is significantly different at $P<0.05$.

Hybrid - Treatment (n=9)	**Young's Modulus of Elasticity 10^3 E (Nmm^{-2})**	**Second Moment of Cross-Sectional Area I (mm^4)**	**Flexural Stiffness 10^5 EI (Nmm^2)**
47-174 Control	2.52±.247 A	98.98±18.75 BC	2.46±.322 BC
47-174 MP	2.02±.333 B	191.60±53.91 A	3.76±.718 A
11-11 Control	2.51±.276 A	81.48±27.24 C	1.98±.563 C
11-11 MP	2.33±.398 AB	121.83±30.26 B	2.76±.593 B

mechanical properties

A significant increase in EI (Nmm^2) was recorded for the MP stems over that of the control in both the 47-174 and the 11-11 hybrids (Table 4). Flexural stiffness between the control stems of the two hybrids was not significantly different, but EI was significantly different between the MP stems of the two hybrids. The 47-174 MP stems showed a larger EI than the 11-11 MP stems (Table 4). I (mm^4) was significantly greater in the MP stems than in the control stems for both hybrids. The control stems of 47-174 did not show a significant difference in I from the control stems of 11-11, whereas the 47-174 MP stems were significantly higher in I than the 11-11 MP stems (Table 4). E (Nmm^{-2}) was significantly decreased in the MP stems over the control for the 47-174 clone (Table 4). The 11-11 stems showed no significant differences between the control and MP. No significant differences in E were found between the control stems, or the MP stems of the two hybrids.

Discussion

The earlier work of Telewski and Jaffe (1981, 1986a and b) and Telewski (1990), which describes a genetic influence on the thigmomorphogenetic effect in conifers, is supported by the data presented here. Their hypothesis that same species plants with different genetic compositions can have a slightly different response to identical mechanical perturbation under similar environmental conditions is also applicable to the hardwood, *Populus* spp. Increased radial growth of the MP stems appeared to be the most significant morphological response to mechanical perturbation for both hybrids (Table 2). The radial growth observed in the flexed stems was asymmetrical; the axis parallel to the direction of mechanical perturbation tended to be longer than the axis perpendicular to MP. In other words, the MP stems took on a slightly elliptical shape, with the long axis in the direction of MP. This type of asymmetrical growth was more apparent in the 47-174 hybrid than in the 11-11 (Table 3). Combining these results with the fact that pre-treatment stems were nearly circular (Table 3) indicates that asymmetrical radial growth, with the long axis in the direction of flexure, can be considered a thigmomorphogenetic response to flexing along a single plane.

Extension growth was not affected significantly by mechanical perturbation (Table 1). When considering the change in height to stem diameter as a ratio, however, a thigmomorphogenetic effect is observed in the MP stems. A significant decrease in the H:D ratio was observed in the flexed stems for both 47-174 and 11-11 after two months of treatment (Table 3). Pre-treatment data indicated no significant differences in H:D between the treatment groups for either hybrid. A decrease in H:D is a representation of the thigmomorphogenetic effect (Telewski and Pruyn, 1997).

Decreased height to stem diameter ratio and the tendency towards an elliptical stem in the MP seedlings (when compared to the control) is considered a significant thigmomorphogenetic response (Jaffe 1980, Telewski and Jaffe, 1986a,b, Telewski and Pruyn 1997). McMahon (1973) discusses an explanation for this response, where the seedling is viewed as a self-supporting column, under loading conditions. Telewski and Jaffe (1986a,b) conclude that many of the observed responses in the wind-stressed, or mechanically perturbed trees are examples of developmental acclimation, which enable them to better withstand bending and swaying stresses by reducing drag and increasing mechanical strength. These conclusions are supported by the constant-strain hypothesis for developing wood in tree stems, described by Wilson and Archer (1979). The basic idea is that the distribution of new wood is regulated by the degree of strain in various regions along the stem. Developing trees tend to conform to a shape that stabilizes the maximum strains along a stem. The tendency toward an elliptical shape and the decrease in the height to stem diameter ratio observed in the MP stems, as compared to the control, represent this directed allocation of wood.

The values generated for E in this experiment (Table 4) are comparable to those published by the United States Department of Agriculture (Forest Products Laboratory, 1987). Young's Modulus for Eastern Cottonwood (*Populus deltoides*) was listed as 6.96 x 10^3 N/mm^2. This value was obtained from green material of small, clear, straight-grained specimens, which were loaded onto simple supports for instron testing. The load administered by the instron was in the center of the stems.

In this study, E was observed to decrease in the MP stems of the 47-174 hybrid (Table 4), which supports the results of Telewski and Jaffe (1986a, b) and Holbrook and Putz (1989). Wilson and Archer (1979) suggest that when E varies in stems as they develop, a different kind of wood is being added. This fact is particularly interesting when comparing E in the MP stems of the 47-174 hybrid to that observed in 11-11. The 47-174 MP stems show a statistically significant decline in E from the control, whereas 11-11 MP stems do not. The 47-174 MP stems appear to respond to the mechanical perturbation to a greater extent than the 11-11. This response may include the formation of wood types that show an increased ability to absorb bending energy under the force of MP.

When considering the parameter of EI, however, this increased flexibility is obscured. EI increases significantly in the MP stems for both 47-174 and 11-11 (Table 4). The increase evident in the structural property (I), which has to do with the amount of material present in the stem, overrides the effect of the decreased material property (E). The net result is that the stems become more rigid in response to mechanical perturbation. This tendency of the structural property to outweigh the material property in the resulting mechanical properties of the organism is evident in the equation (2) for I. The stem radius scales to the fourth power in equation (2), whereas the lengths along the stem (a and L) only scale to the second power in equation (1). The effect of the observed increase in the radial growth of the MP stems is thus revealed in the stem's mechanical properties. Just a slight increase in stem diameter results in a significant increase in the stem's flexural stiffness. The tendency for mechanically perturbed and wind-stressed stems to increase in flexural stiffness was also observed by Telewski and Jaffe (1986a, b). An increase in EI is another example of the thigmomorphogenetic effect.

Harrington and DeBell (1996) defined specific phenotypes, representing extremes of wind-firmness, for the two *Populus* hybrids, 47-174 and 11-11. 47-174 was described as the most resistant hybrid to wind toppling, with the highest rate of lower stem taper. These phenotypic observations were supported by this study. The 47-174 control and MP stems had higher ratios for change in height to stem diameter (ΔH:D) than the respective stems of 11-11 (Table 3), which indicates a stronger stem taper for 47-174. 47-174 MP stems also showed higher values of EI the 11-11 MP stems (Table 4) indicating that the 47-174 hybrids have stems that are more rigid than 11-11. These results support Harrington and DeBell's (1996) observation that 47-174 trees were the most resistant to wind throw, whereas 11-11 trees were the least resistant. Adding more wood to the stem's diameter, which results in an increased I, strengthens the stem. Decreased E weakens the wood in the stem; however, this weakening is more than compensated by the increase in I, resulting in an increase in EI. More of this weaker wood is used to create a stem that has equal loading capacity as compared to a stem with a higher E.

In conclusion, it appears that the two *Populus* hybrids, 47-174 and 11-11, posses hybrid-specific differences in morphology and their mechanical properties. These differences directly effect the hybrid's tolerance of mechanical perturbation. Some interesting possibilities for further research include the anatomical and biochemical analyses of the cross-sections of these stems. Changes in the material and structural properties of the wood in the 47-174 MP stems could then be explained on the cellular level.

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CHAPTER THREE: Anatomical and Biochemical Responses of Two *Populus* Hybrids to Mechanical Stress

Introduction

Thigmomorphogenesis is defined as the morphgenetic response of plants to wind and other mechanical perturbations (Jaffe 1973). A decrease in stem elongation and an increase in radial growth (Jaffe 1973) characterize the thigmomorphogenetic response. This type of response has been discussed extensively in gymnosperms and herbaceous angiosperms.

At the tissue level the increased radial growth has been documented as typically asymmetrical in coniferous species. This type of growth is the result of an increased number of tracheids in the direction of flexure, or on the leeward side of the stem (Larson 1965, Bannan and Bindra 1970, Burton and Smith 1972, Quirk et al. 1975, Telewski and Jaffe 1981, 1986a, Telewski 1989). The tracheids that occur in the long axis of these asymmetrical stems are shorter than those in other regions of the stem, or those in unstressed stems (Bannan and Bindra 1970, Telewski and Jaffe 1986a,b). Record of anatomical changes in response to anatomical stress in angiosperm species is less abundant. One study of flexed *Liquidamber styraciflua* revealed a reduction in vessel element length and diameter as compared to the unstressed controls (Neel and Harris 1971).

These changes in anatomy and morphology in mechanically perturbed, or wind-stressed stems have significant effects on the mechanical stability of these trees. Biomechanically, the flexed stems in *Abies fraseri* and *Pinus taeda* show an increase in the property of flexural stiffness (EI), which is the product of Young's elastic modulus (E) and the second moment of cross-sectional area (I) (Telewski and Jaffe 1986a,b). While I increases because of the increase in radial growth in flexed stems, E actually decreases. This decrease in E is outweighed by the increase in I, however, which scales to the fourth power with stem radius. A reduction in E gives the stem a decreased ability to absorb the bending energy induced by flexure. An overall increase in EI strengthens the stem, reducing its susceptibility to wind-snap. A similar trend was elucidated in angiosperms. A lower value of E and a higher value for I in free-swaying stems of *Liquidambar styraciflua* suggest an increase in flexural stiffness from the constrained control stems (Holbrook and Putz 1989). However, whole-tree flexibility, which is measured as deflection/force (in radian/N), of the free-swaying stems was not significantly different from the control.

These changes in the mechanical properties of flexed stems should also be explainable in terms of the biochemistry of the woody tissues. Surprisingly, significant changes in the biochemistry of mechanically perturbed woody stems have not been identified. When lignin was extracted from the tissue of stressed *Pinus taeda* stems, no significant differences from the controls were identified (Telewski and Jaffe 1981). In *Bryonia diocia* lignification was accelerated in response to rubbing (DeJaegher et al. 1985). Grasping the stem at designated place and rubbing it with the thumb and fore-finger is a method used to initiate and examine thigmomorphogenesis. DeJaegher and Boyer (1987) later confirmed these results by counteracting the rubbing-induced lignification using specific inhibitors for cinnamyl alcohol dehydrogenase (a key enzyme in the lignin biosynthesis pathway). A record of biochemical analyses of flexed versus control stems of woody angiosperm species is currently not available in the literature. A thorough biochemical investigation comparing mechanically perturbed or wind-stressed stems to non-stressed controls is crucial to the understanding of plant responses to flexure stress.

For this investigation, two hybrids were selected based on characteristics of wind-firmness described by Harrington and DeBell (1996). A cross between *Populus deltoides* and *Populus trichocarpa* resulted in hybrids that exhibited differences with regard to wind tolerance. The hybrid, 11-11, was identified as wind intolerant and more susceptible to wind toppling and wind throw. Among the various hybrids in the study, 11-11 had the highest amount of above-ground biomass per unit of root area, and the highest ratio of above-ground biomass to cross-sectional area of the mean root (Harrington and DeBell 1996). 11-11 had the highest value for stem slenderness when expressed as height divided by root-collar diameter. It also had the lowest rate of stem taper from groundline. The study also identified a wind tolerant hybrid, 47-174. In comparison with the other hybrids from the study, the wind-firm hybrid had the highest rates of lower stem taper, the lowest amounts of above-ground biomass per unit of cross-sectional root area, and the largest roots (Harrington and DeBell, 1996).

The biomechanical and morphological responses of these two poplar hybrids, 11-11 and 47-174, were described in Chapter Two of this paper. The purpose of this chapter is to determine whether there is an anatomical and biochemical explanation for the mechanical and morphological responses documented.

Materials and methods

Culture of the plants

The trees used in this study were two hybrids from the F_1s of a cross between *Populus trichcarpa* and *Populus deltoides*. One hybrid, 47-174, is designated a wind tolerant hybrid and the other, 11-11, is considered an intolerant variety, based on the field studies of Harrington and DeBell (1996). Stem cuttings of these plants have been successfully rooted during the past two years in a nursery at MSU. The rooted cuttings were transplanted into 7.3 x 7.3 x 22.8cm Utility Band pots (Anderson Die and Manufacturing Company, 2425 S.E. Moores St., Portland, OR, U.S.A.). The growth medium used for these plants was a combination (in a ratio of 1:1) of a high porosity professional planting mix, supplied by Baccto (Michigan Peat Co., P.O.Box 980129, Houston, TX 77098) and a recycled and sterilized sandy mix, supplied by the MSU greenhouse. After sprouting was initiated, the sprouted cuttings were transplanted twice with growth. The first transplant was to an 8L pot and the final, to an 18l pot. These cuttings were cultivated in a greenhouse under photoperiods ranging from 12 to 14 hours. Diurnal temperatures were maintained at 25°C. The original stem cuttings were left to grow for two weeks undisturbed after the final transplanting. During this time, new stem shoots were initiated for each cutting. At the end of two weeks, one leader stem was selected from every cutting for uniform height and stem width. Any other shoot growth from the main stem was removed. Each selected leader stem was then staked.

Eighteen individuals of each hybrid were used. There were two treatments: control and mechanical perturbation (20 flexures). Nine of the 47-174 hybrids were control, and the other nine were flexed mechanically in the NE to SW direction. The same design was used for the 11-11 hybrid. The flexure treatments were administered once daily for the months of July and August of 1996. Before treatments began, a point of flexure was chosen for the stems. The flexure point is the place on the stem that is grasped each time for treatment with the thumb and forefinger. The other hand is used to shake the stem by grasping the plant at several centimeters above the flexure point. This flexure point was designated as the point just above the fourth node from the base of each leader stem. The daily treatment consisted of gently grasping the stem at the flexure point and bending it back and forth, not exceeding more than 45° from the original vertical position of the stem. After treatment, the stem was returned to the vertical position and restaked.

Over the course of the experiment, pesticides were applied three times. Azatin and Talstar were used at a rate of 2-4 ml per 4 liters of water. This chemical spray was applied to the subject population twice within two weeks time for the purpose of removing aphids. Avid was applied once in spray form (rate of 1ml per 4 liters of water) for white fly control.

The seedlings were fertilized using Peters 20-20-20 (Scotts-sierra Horticultural Products Co., 14111 Scotslawn Rd., Marysville, OH 43041)once after one month of treatment, using a Miracle-Gro Siphonex Brass Siphon Mixer (Scotts Miracle-Gro Products, Inc., Port Washington, NY 11050, U.S.A.) to the greenhouse garden hose for application. The fertilizer concentration applied to the poplars was 465ppm, or 30 grams per 4 Liters of water.

Anatomical measurements

After harvesting, the stem cuttings were stored in a 30% ethanol solution. A sliding microtome was then used to make transverse and tangential sections, 40um thick, in the designated region of flexure stress. Sections were tied to microscope slides (VWR Scientific, Inc., P.O. Box 3200, San Francisco, CA 94119) and stained following the safranin/fast-green staining procedure from Berlyn and Miksche (1976).

Phloroglucinol-HCl (Hammerschmidt 1984) and Chlorazol-Black E (Robards and Purvis 1964) stains were also used on the mounted cross sections for tissue analysis. They were mainly used to corroborate the safranin/fast-green staining.

A Zeiss 10 Laser Scanning Confocal Microscope (CLSM) (Carl Zeiss, Inc.) was used to visualize the fibers in the safranin/fast-green stained cross-sections of both hybrids. It was especially useful in visualizing gelatinous fibers. The external, dual-line, argon ion laser, emitting at 488nm (blue) was used as a light source. The images were viewed on a monitor screen and photographed using a Matrix Multicolor computerized camera unit for 35mm black and white prints.

From safranin/fast-green stained cross-sections of five of the nine replicates for both treatments of each hybrid the vessel lumen areas were calculated using a computer-based system for image analysis of digitized images. The measurements were taken under 20X magnification on a Nikon Labophot (Japan) Microscope. Two areas were chosen on both sides of the pith along the same axis in all stems, in the MP stems this axis was in the direction of flexure. The total area scanned was 0.50 mm^2. A DAGE MTI VE 1000 camera and an HR-1000 Multiscan Monitor was used to capture and digitize the microscopic images (DAGE MTI, Inc. Precision Video, 701 N. Roeske Ave., Michigan City, IN 46360, U.S.A.). Data from a NIH image analyzer program was entered into Microsoft Excel - version 5.1 for the purpose of calculating the percentage of vessel lumen area per mm^2 and the average vessel lumen area (um^2). For each treatment, the distribution of vessel areas was tested for Normalcy using the Kolmogrov-Smirnov Normality Test, MiniTab - version 11.12. The populations were tested for significant differences within treatment groups using ANOVA in SAS - version 6.11. A multiple comparison among the means of each treatment group (the Duncan's Multiple Range Test) was used to determine which treatments were significantly different.

Biochemical Analyses

Lignin analysis by thioglycolic acid procedure: The LTGA procedure was adapted from Hammerschmidt (1984). For each hybrid, five of the nine replicate stems were selected from each treatment. Keeping the replicates separate and labeled, 2-3cm segments were cut from the region on the stem designated as the flexure point. The segments were then vacuum-dried at 20°C, and removed of their periderms. A Wiley mill was used to grind each stem to a powder that passed through a 20 mesh screen; 40mg of each ground stem was placed in a standard test tube with 0.5ml of thioglycolic acid (Sigma Chemical Co., St. Louis, MO) and 2ml of 2N HCl. A glass marble was placed over each tube to serve as stoppers to prevent evaporation of the solution during heating.

The tubes were then heated for 4h at 95°C. The solids were collected via suction filtration using a Buchner funnel with a fritted disc and a side-arm flask; the supernatant was discarded. The solids were then washed three times with 5ml of distilled water. Five ml of 0.5N NaOH was added to each tube. The tubes were kept overnight in order to solubilize the ligninthioglycolic acid (LTGA). The solids were again separated by suction filtration and washed two times with 2ml of distilled water. The solids were discarded, and the washes were added to the supernatant along with 1ml of concentrated HCl. This mixture was stored at 4°C for 4h in order to enhance precipitation of LTGA. The final precipitate was collected by centrifugation, and washed twice by resuspension of the pellet in 2ml of 0.1N HCl, followed by centrifugation. The pellet was then dissolved in 0.5N NaOH to a final volume of 5ml. Before measuring the absorbency of the solutions, they were centrifuged to remove any insoluble material remaining. The absorption of the solvent was 280nm.

In order to determine final concentrations for each treatment's absorbency, a standard curve was generated. Aliquots of 1ml were drawn from each replication for each treatment and combined into one test tube. Concentrated HCl (5ml) was added, and the mixture was stored overnight. A precipitate was collected by centrifugation and washed twice by resuspending the pellet in 2ml of distilled water, followed by recentrifugation. The final pellet was vacuum dried overnight and then weighed. The pellet was dissolved in 5ml of .5N NaOH. A serial dilution was made of the solution. The absorbency for each dilution was measured at 280nm under UV. Plotting Absorbency (280nm) versus Concentration (mg/ml) created a standard curve.

The populations were tested for significant differences within treatment groups using ANOVA in SAS - version 6.11. A multiple comparison among the means of each treatment group (the Duncan's Multiple Range Test) was used to determine which treatments were significantly different.

Lignin analysis by Pyrolysis GC-MS: The method was adapted from Ni et al. (1996) and executed by R. Ted Jeo and Dr. Hans Jung (U.S. Department of Agriculture, University of Minnesota). A pyrolysis power generator (Pyroprobe 1000, CDS Analytical, Oxford, PA) pyrolyzed the ground and dried tissue (100-200ug) in a quartz tube. The pyrolysis lasted for 10 seconds at 700°C (>20°C/ms ramp) using helium as a carrier gas with a mean linear velocity of 15.4cm/min. The sample was partitioned on a capillary dimethylpolysiloxane column (DB-1, J&W Scientific, Folsom, CA) (0.25mm X 60m x1.0um) and analyzed on a Hewlet-Packard (model

6890, series II) gas chromatograph in splitless mode. The initial temperature was set at 50°C for two minutes, and then increased at a rate of 4°C/min until a final temperature of 275°C was reached. The total run time was 63 minutes. Compounds were detected with a mass selective detector (model 5971A, Hewlett-Packard) and data were collected and analyzed using the ChemStation software in DOS (Hewlett-Packard).

The populations were tested for significant differences within treatment groups using ANOVA in SAS - version 6.11. A multiple comparison among the means of each treatment group (the Duncan's Multiple Range Test) was used to determine which treatments were significantly different.

Lignin analysis by cupric oxide oxidation: For each hybrid, five of the nine replicates were combined for each treatment, control and MP. A 1cm segment was cut from the region on the stems designated as the flexure point. The periderm of each segment was removed. The remaining tissue was then ground into a fine powder (20 mesh) using a Wiley mill. The ground tissue was then prepared so that it was extractive-free. Fifty mg of tissue was placed in fritted glass extraction thimbles in a Soxhlet extractor. An extraction was performed with 95% EtOH and toluene in a ratio of 1:2 for a period of 8h. Two subsequent extractions with 95% EtOH and dH_2O

were performed for 8h and 4h, respectively. The extractive-free method was adapted from J. Dean (1997).

The CuO oxidation procedure was adapted from Hammerschmidt (1984). The powdered, extractive-free material was reweighed and then placed into a 10ml Teflon-lined digestion bomb along with 680mg of $CuSO_4$ (Baker Chemical Co., Phillipsburg, N.J. 08865, U.S.A.) and 5ml of 3N NaOH. The bomb was sealed and heated at 180°C for 2.5h. After cooling in an ice bath for 20minutes, the contents of the bomb were transferred to a conical centrifuge tube. The inside of the bomb was rinsed out twice with 1ml of distilled water. The solids were removed by centrifugation (10,000g, 5min) and the supernatant was transferred to a large test tube. The solids were washed and resuspended in 2ml of dH_2O, followed by centrifugation. The resulting supernatant was added to the test tube. The combined supernatants were adjusted to pH 3 with 5ml of 6N HCl. The supernatant was then extracted three times using 5ml of diethyl ether. The ether extracts were partitioned against an equal volume of freshly prepared 1% $NaHCO_3$, which removed the phenolic acids. After removing the aqueous layer, the ether was then evaporated to dryness under a stream of nitrogen. The residue was dissolved in 100ul of methanol.

The three standard aldehydes, vanillin, syringaldehyde, and p-hydroxybenzaldehyde (Sigma Chemical Co., P.O. Box 140508, St. Louis, MO 63178, U.S.A.), were used for comparison of the aldehydes in the unknown samples. The standards were dissolved in 1ml of methanol. The unknown and the known samples were micro-pipetted in 50μl aliquots onto a Redi Plate/Silica Gel G (Fisher Scientific Co., Fairlawn, N.J. U.S.A.). The aldehydes were separated via thin layer chromatography using the solvents, toluene - ethylacetate - glacial acetic acid (ratio 1:1:1).

Results

Anatomical Measurements

The average vessel lumen area was lower in the MP stems than the control of both 47-174 and 11-11, however, the difference was not significant (Table 1). The 11-11 stems showed a pattern to decrease in average vessel lumen area from 47-174, with a significant decrease in the 11-11 MP stems from the 47-174 MP stems (Table 1). The proportion of the xylem cross-sectional area that was vessel lumen dropped significantly in the MP stems as compared to the control for both hybrids (Table 1). There were no significant differences between the hybrids in percent lumen area (Table 1). The MP stems of both 47-174 and 11-11 showed a decrease in the number of vessels per 1mm^2 of xylem tissue; the difference, however, was insignificant (Table 1). The vessel density (number of vessels per 1mm^2) was greater in the 11-11 stems than 47-174 for both treatments (Table 1).

Table 1: **Vessel Lumen Measurements** of *Populus trichocarpa* x *P. deltoides* hybrids after two month's exposure to flexure treatments of 20X per day. Measurements were taken along the same axis in all stems; in MP stems this was the axis of flexure. For each measurement, a mean (±SE) followed by a different letter is significantly different at $P<0.05$.

Hybrid – Treatment (n=5)	**Average Vessel Area (um^2)**	**% Lumen Area**	**No. of Vessels per mm^2**
47-174 Control	1520.9 ± 85.3 A	0.224 ± 0.0147 A	135.4 ± 5.59 BC
47-174 MP	1434.7 ± 88.0 A	0.184 ± 0.0068 B	117.6 ± 4.16 C
11-11 Control	1327.7 ± 59.3 AB	0.234 ± 0.0075 A	158.0 ± 9.93 A
11-11 MP	1115.4 ± 48.7 B	0.186 ± 0.0040 B	149.3 ± 7.16 AB

In both treatments from each hybrid, positive staining for lignin was observed using the stains safranin and phloroglucinol-HCl. All the sections also showed positive staining for cellulose with the Fast-Green and Chlorozol Black E stains.

Using the CLSM on the safranin/fast-green stained cross sections, the fibers of both hybrids were visualized. Figures 1-4 show cross sections of xylem tissue under 1200X magnification for the control and MP stems of both hybrids using CLSM. Gelatinous fibers were identified in both control and MP stems of both hybrids (Figures 5-8).

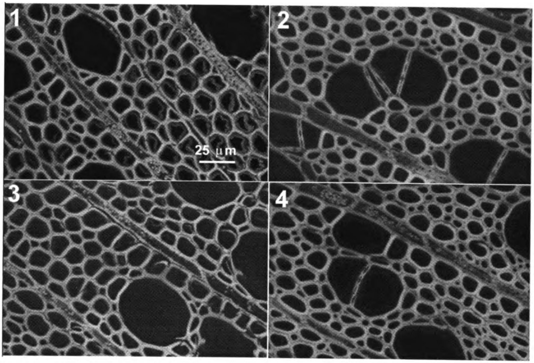
Figures 1-4 are photographs of 40µm cross sections of stems from both *Populus* hybrids, visualized using fluorescence (480nm, blue laser) in confocal scanning laser microscopy.

Figure 1 - 11-11 Mechanically Perturbed Stem Cross Section at the Flexure Point

Figure 2 - 11-11 Control Stem Cross Section at Position Equidistant from the Base of the Terminal Shoot as Compared to Flexure Treatment

Figure 3 - 47-174 Mechanically Perturbed Stem Cross Section at the Flexure Point

Figure 4 - 47-174 Control Stem Cross Section at Position Equidistant from the Base of the Terminal Shoot as Compared to Flexure Treatment


1
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25 µm
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4

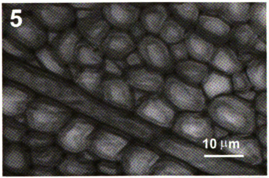
Figures 5-8 are photographs of 40μm cross sections of stems from both *Populus* hybrids. The images are laser transmitted (480nm, blue laser) in confocal scanning laser microscopy.

Figure 5 - 11-11 Mechanically Perturbed Stem Cross Section,
Gelatinous Fibers

Figure 6 - 11-11 Control Stem Cross Section, Gelatinous Fibers

Figure 7 - 47-174 Mechanically Perturbed Stem Cross Section,
Gelatinous Fibers

Figure 8 - 47-174 Control Stem Cross Section, Gelatinous Fibers



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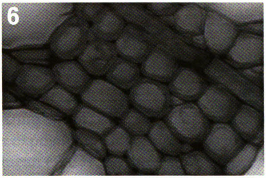

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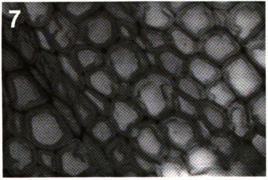

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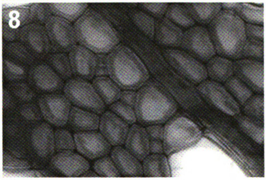

8

Biochemical Analysis

The thioglycolic acid analysis resulted in a significantly lower yield of ligninthioglycolic acid (LTGA) in the MP stems of 47-174 over the control. The 11-11 MP no significant difference in LTGA yields. There were no significant differences in the amount of LTGA between the two hybrids, except for the drop in the 47-174 MP stems, which was significantly lower than all other stems (Table 2).

Table 2: **LTGA Yields,** (mg LTGA/mg wood) of *Populus trichocarpa* x *P. deltoides* hybrids after two month's exposure to flexure treatments of 20X per day. For each measurement, a mean (±SD) followed by a different letter is significantly different at $P<0.05$.

Hybrid - Treatment (n=5)	**mg LTGA / mg wood**
47-174 – Control	0.393 ± 0.0311 A
47-174 – MP	0.298 ± 0.0478 B
11-11 – Control	0.361 ± 0.0556 A
11-11 – MP	0.357 ± 0.0224 A

The pyrolysis-GC/MS analysis of lignin revealed a slight, yet insignificant decrease in the ratio of syringyl to guaiacyl units in the MP stems over that of the control in the 47-174 hybrid (Table 3). However, this ratio increased insignificantly in the 11-11 MP stems. The MP stems of the 11-11 hybrid showed significantly lower levels from the control in 4-

vinylguiacol, which is derived mainly from ferulic acid (Table 3). There was no significant difference between the Control and MP of 47-174 for 4-vinylguaiacol units. 4-vinylphenol, derived from p-coumaric acid, was not found in any of the samples. There were no significant differences between the two hybrids (Table 3).

Table 3: **Pyrolysis-GC/MS Analysis** of *Populus trichocarpa* x *P. deltoides* hybrids after two month's exposure to flexure treatments of 20X per day. For each measurement, a mean (±SD) followed by a different letter is significantly different at $P<0.05$. Columns without letters showed no significant differences.

Hybrid – Treatment (n=5)	Guaiacyl Units	Syringyl Units	Syringyl / Guaiacyl ratio	4-vinyl Guaiacol Units
47-174 - Control	7.91±2.33	17.9±3.00	2.33±0.310	1.73±0.178 AB
47-174 - MP	7.65±1.90	17.5±4.23	2.30±0.343	1.70±0.232 AB
11-11 - Control	8.41±2.63	18.4±7.64	2.16±0.202	2.06±0.375 A
11-11 - MP	6.91±1.32	16.2±2.29	2.39±0.302	1.59±0.220 B

Quantitative analysis of the CuO oxidation has not yet been conducted. A qualitative analysis reveals that p-hydroxy benzaldehyde (corresponding to p-coumaryl alcohol) was detected in very low amounts in all samples (Figure 9). Detection of this aldehyde was especially low in the

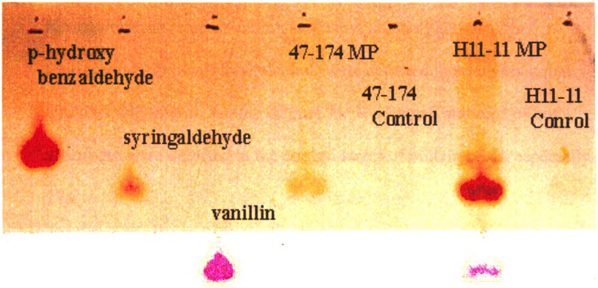

Figure 9: Separation by thin layer chromatography of CuO oxidation produced phenolic aldehydes

control stems of both hybrids, where it does not appear at all (Figure 9). Syringaldehyde (corresponding to sinapyl alcohol) and vanillin (corresponding to coniferyl alcohol) were detected in relatively equal amounts within each sample (Figure 9). Very small amounts of both aldehydes were detected in the control stems of both hybrids, especially 47-174.

Discussion

The drop in percent vessel lumen area per mm^2 of xylem tissue in the MP stems of both *Populus* hybrids (Table 1) is an example of the thigmomorphgenetic response and is consistent with the earlier work in coniferous species. Telewski and Jaffe (1986a, b) recorded a significant increase in the number of tracheids along a radial file in the direction of flexure in mechanically perturbed stems of *Pinus taeda* and *Abies fraseri*. In *Abies fraseri* they noted a decrease in tracheid lumen cross-sectional area along the flexed axis of the stem (Telewski and Jaffe 1986a). The results in *Populus* are also in agreement with the findings of Neel and Harris (1971) in the angiosperm, *Liquidambar*. They noted that moderate shaking of these trees resulted in a significant decrease in vessel member diameters as compared to the unshaken controls.

The 11-11 stems showed a trend of decreased average vessel lumen area as compared to the 47-174 stems and increased number of vessels per um^2 of xylem tissue (Table 1). In other words, the vessels in 11-11 stems tend to be smaller on average, yet there are more of them. This trend is evident from the results for percent lumen area, which show no significant differences between the two hybrids. These differences in vessel lumen area and vessel density potentially have significant impact on the biomechanical properties of the stem. At the scale of a growth ring, denser wood (less vessels) has the ability to bear more stress than less dense wood if both receive the same strain (Gartner 1995). The 47-174 stems, therefore, are indicated as more capable to bear stress than 11-11.

The Phloroglucinol-HCl staining revealed that lignin was present in the fiber, vessel, and ray cell walls of all stems. Gelatinous fibers were identified as having a thin outline of reddish pink stain in the cell wall, with a loose, unstained thicker layer, found just inside. The safranin/fast-green and Chlorazol black staining confirmed that there were gelatinous fibers in the control and MP stems of both hybrids. Gelatinous fibers stain a blue-green color when the safranin/fast-green method is used, and they stain black when the chlorazol black method is used. Gelatinous fibers were also visualized using CLSM (Figures 5-8).

The response to the mechanical stress in the *Populus* hybrids also includes changes in the lignin content of the xylem tissues. Results from the thioglycolic acid extraction suggest that total LTGA lignin decreased in the MP stems of 47-174 (Table 2). These results are contrary to the findings in *Bryonia doica*, where lignification was shown to increase in mechanically stressed stems (DeJaegher et al. 1985, DeJaegher and Boyer 1987). The implications of the current findings are interesting in light of the fact that total lignin content in tension wood is reduced from that in normal wood, if represented as a percentage of the total wood, including additional cellulose in the gelatinous fibers (Creber 1975, Panshin and DeZeeuw 1980). Tension wood is the reaction wood that forms on the upper sides of branches, or leaning stems in angiosperms (Creber 1975). Matteck and Kubler (1995) state that the decrease in lignin typical of tension wood serves to increase the ability of such stems to yield under bending stresses; in other words, tension wood is ideally adapted to sustain tensile stress. The decrease in lignin in tension wood is usually attributed to the presence of a gelatinous layer (G-layer) within the secondary wall layers of its fibers. The G-layer is composed primarily of cellulose, which explains its provision of tensile strength to a branch, or leaning stem. The reduction of LTGA lignin in the MP stems of the 47-174 hybrid in this study suggests that flexure wood shares some characteristics of tension wood.

The xylem tissue in these poplar stems was also analyzed in terms of its lignin composition. The Pyrolysis-GC/MS analysis revealed that there were at least twice as many syringyl as guaiacyl units released from the lignin in all the stems (Table 3). The analysis also suggests a tendency in the stressed 47-174 stems to decrease in their ratio of syringyl to guaiacyl units, whereas the 11-11 MP stems showed a slight increase in this ratio (Table 3). These results are roughly supported by a qualitative analysis of the CuO oxidation. The syringyl and guaiacyl units appear to be released such that there is more syringyl than guaiacyl units in each stem (Figure 9). This is especially evident for the MP stems. These changes in the monolignol units of lignin may be representative of changes in the entire lignin structure of the stems. Dean and Eriksson (1992) report on the various studies that reveal the wide variation in the guaiacyl and syringyl residue composition of lignins from gymnosperms, angiosperms, and grasses. Dean and Eriksson (1992) also note that physical and environmental stresses can vary this composition as well. An example is in compression wood, which has a higher concentration of p-coumaryl than guaiacyl units in its lignin (Timell 1986).

Another interesting aspect of the pyrolysis-GC/MS analysis is the significant decrease in the 4-vinyl guaiacol units (analogous to ferulic acid) of the 11-11 MP stems (Table 3). Ferulic acid is usually not considered to be one of the main lignin components in dicot angiosperms (Dean and Eriksson 1992, Dean 1997, Sarkanen 1963). It is difficult to know, at this point, if it is an actual component of lignin; or if it is merely a ubiquitous phenolic compound within the xylem tissue, not actually associated with the lignin. Buchanan (1963) and Schubert (1965) do not rule out the possibility that ferulic acid is an actual component of lignin. The tissue for the Pyrolysis-GC/MS analysis was not extractive-free, which allows for the possibility that the ferulic acid units detected were of the loose, ubiquitous phenolics in the xylem tissue. The significant difference observed in the 11-11 MP stems over the control, however, gives cause for a more thorough investigation in this area.

Conclusions and Future Research

The anatomical and biochemical analysis conducted on the *Populus* hybrids, 47-174 and 11-11, provides an interesting perspective for the morphological and biomechanical results from Chapter Two of this paper. When compared to the control, both MP hybrids show a significant increase in radial growth and a tendency toward an elliptical stem in the direction of flexure (Tables 2 and 3, Chapter Two). The anatomical analysis resulted in a tendency to decrease in vessel lumen area per um^2 in the direction of flexure of the MP stems of both hybrids (Table 1). A logical conclusion, therefore, is that the documented increase in radial growth is due to an increase in the number of fibers in the xylem tissue. This assumption should, of course, be tested by further investigation of fiber area. Measurements of fiber cell wall thickness in the direction of flexure would also provide useful information.

Biomechanically, the flexural stiffness (EI) increased in the MP stems of both hybrids compared to the control, whereas Young's Elastic Modulus (E) decreased in these stems (Table 4, Chapter Two). This same pattern of an increase in EI and a decrease in E was identified when comparing 47-174 stems to 11-11 stems for control and MP (Table 4, Chapter Two). These results suggest that the observed increase in EI arises from the increase in I and not E. Stems with increased EI are less resistant to stem snap, or mechanical failure (Telewski 1986a, b, Wainwright et al. 1976, Wilson and Archer 1979). The anatomical design of the MP stems and of the 47-174 hybrid is potentially more suited for mechanical stress than the controls, and the 11-11 hybrid respectively. These data support Harrington and DeBell's (1996) description of 47-174 as the most resistant, and of 11-11 as the least resistant hybrid to wind toppling.

One possible explanation for the decrease in E in the MP stems is their decrease in vessel lumen area per mm^2 xylem as compared to the control. Less vessel lumen area indicates increased area of fiber cell walls. The decrease of E in the MP stems of both hybrids suggests a decrease in the material strength in the stem. The question is then raised that if more fibers are being added than vessels by the stem, why is the material strength decreasing? A potential explanation for the decrease in E is the presence of gelatinous fibers. Gelatinous fibers function to increase bending ability in the stem (Matteck and Kubler 1995). MP stems of both hybrids show a trend towards a decreased Young's Modulus (E), with 47-174 MP stems lower than 11-11. Although a drop in E indicates decreased material strength, it also suggests increased flexibility in the stem (Wainright et al. 1976), which could be attributed to the gelatinous fibers within the stems.

Gelatinous fibers were identified in all stems (Figures 5-8). An interesting next step to this analysis would be to calculate the percentage of gelatinous fibers in the MP stems versus the control and between the two hybrids. This procedure would provide a clearer picture of how many fibers are contributing to material stiffness (increase E) in the stem, versus how many gelatinous fibers are serving to weaken the stem (decrease E). Combing these data with the vessel density data from Table 1 could explain more thoroughly the biomechanical properties on the anatomical level.

The anatomical analysis of these poplar hybrids raises the question as to whether these poplar stems are forming tension wood in response to flexure. The presence of gelatinous fibers in xylem tissue is a characteristic of tension wood (Panshin and DeZeeuw 1980), but they also have been identified in stems grown rapidly under intensive management (Isebrands and Bensend 1972). Gelatinous fibers were identified in the control stems as well as MP (Figures 5-8). Tension wood has also been shown to have vessels with decreased lumen diameters, and fewer vessels per mm^2 of xylem tissue (Panshin and DeZeeuw 1980). This trend was evident in the MP stems of both hybrids as compared to the control (Table 1). The comparison of 11-11 stems to 47-174 does not reveal this trend; instead, 11-11 has smaller vessels, but an increased vessel density. A more thorough anatomical analysis is necessary in order to characterize the wood that is forming in response to flexure in these stems. This "flexure wood" could then be described in terms of its similarity to tension wood and/or normal wood.

Another interesting extension of this study would be to calculate the number and area of the ray cells in the MP stems as compared to the control and between the two hybrids. Matteck and Kubler (1995) state that rays have the effect of initial defects, or "crack starters". These rays decrease the transverse strength of the wood. Changes in the rays have been documented in tension wood of *Populus monilifera.* The rays were fewer in number, but composed of more cells with a greater average height (Kučera and Nečesaný 1970). Because the MP stems of both hybrids show a decrease in E, it is probable that the number and area of rays would increase in the MP stems. The same trend would potentially be identified in the 47-174 stems as compared to 11-11. It would be interesting to determine if the changes in the rays of these hybrids were similar to those found in the tension wood of *P. monilifera* (Kučera and Nečesaný 1970).

The changes in lignin content and composition in the MP stems of both hybrids might explain the decrease in Young's modulus (E). Wilson and Archer (1979) report that changes in E suggest an alteration in the kind of material in the stem. 47-174 MP stems had lower lignin content than the controls and the 11-11MP stems (Table 2). These results indicate that the 47-174 MP stems have potentially higher levels of cellulose than the controls, or the 11-11 MPs. This biochemical difference in the 47-174 MP stems may explain their significantly reduced values of E. Clearly, the biochemical analysis of these *Populus* hybrids is in its early stages. The CuO oxidation should be analyzed quantitatively, and other quantitative extractive methods should be used to verify the thioglycolic acid results. Dean (1997) provides an excellent summary of methods in lignin analysis. The biochemical analysis should also be confirmed anatomically using autofluorescence under UV excitation and confocal laser scanning microscopy to quantify the lignin spatially within the xylem fibers.

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