# An Investigation into Mechanisms of Shoot Bending in a Clone of *Populus tremuloides* Exhibiting 'Crooked' Architecture

BY

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In Partial Fulfillment of the Requirements
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MASTER OF SCIENCE

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## ABSTRACT

Linden, Ashley Wade. M.Sc., The University of Manitoba, February, 2006. An Investigation into Mechanisms of Shoot Bending in a Clone of *Populus tremuloides* Exhibiting 'Crooked' Architecture. Major Professor; W. R. Remphrey.

*Populus tremuloides* Michx. (trembling aspen) is a tree species native to much of North America, characterized by an excurrent crown with horizontal to ascending branches and a dominant terminal leader. An unusual clone of trembling aspen was discovered in the 1940s near Hafford, Saskatchewan. This clone demonstrates abnormal crown morphology, in which vigorous shoots bend down, ultimately leading to an overall twisted or crooked appearance. The objectives of the present study were to investigate the mechanism of shoot bending by (1) characterizing the process and timing of bending, (2) evaluating structural aspects of developing wild-type and crooked aspen shoots, and (3) comparing anatomical features of bending shoots with wild-type shoots. L-system reconstruction models of 3-D digitized shoot development revealed dramatic bending midway through the growing season. Morphological analyses revealed that crooked aspen shoots had greater taper compared to the wild-type, typically known to create shoots resist deflection and bending. However, preliminary strength analyses indicated that crooked aspen shoots were less rigid, with smaller values of Young's modulus compared to wild-type shoots. Anatomical investigations revealed differences in several structural tissues between developing wild-type and crooked aspen shoots, and differences within crooked aspen shoots. Primary phloem fibres on the upper side of bending shoots maintained relatively large lumens while those on the lower side were fully

lignified, similar to those of mature vertically oriented wild-type leader shoots. These differences may result in differential extension growth early in development, and/or uneven mechanical support later on, ultimately resulting in bending due to self-weight. Gelatinous fibres (G-fibres), characteristic of tension wood (TW), were found throughout older wild-type and vertically oriented crooked aspen shoots; however, G-fibres were only found on the lower side of crooked aspen shoots. These lateral differences could have contributed to shoot bending by actively bending shoots downwards, or lack of TW on the upper side may not have prevented biomechanical bending from self weight. Nevertheless, shoot bending stops at the end of the growing season, suggesting that the mechanisms involved in creating bent shoots are only functional during the first growing season.

# 1.0 INTRODUCTION

Trees have the distinction as being the largest and oldest living organisms on earth (Zimmermann & Brown 1971), presenting incredibly complex, yet organized, structural patterns. The development of these multifaceted patterns continually fascinates biologists, engineers, mathematicians, and the like. No doubt, branching patterns evolved as a mechanism to obtain resources, such as light, while the plant is fixed in the same location for its entire life. The arrangement of above-ground stems is known to be somewhat genetically predetermined (Hallé et al. 1978; Ceulemans et al. 1990); however, plants do have the ability to react to environmental and mechanical stimuli (Edelin & Atger 1994; Fournier et al. 1994; Wilson 1998; Jourez et al. 2001), reorienting stems accordingly.

Several complex mechanisms are involved in arranging stems; including, for a large part, the formation of secondary tissues. These tissues are added in a radial manner while the stem expands longitudinally, and are capable of providing significant structural support. Also, anomalous structures within secondary tissues are known to function in creating bending forces that are capable of changing stem orientation (Wilson & Archer 1977; Fisher & Stevenson 1981; Jourez & Avella-Shaw 2003). Studying secondary development in woody plants is essential to better understand the mechanisms involved in creating and maintaining specific forms in plants (Kervella et al. 1994; Chaffey 2002a).

Scientifically, *Populus* is among the most studied and most written about tree genera (Dickmann et al. 2001), and is commonly used as a model system for the study of tree secondary development and anatomy (Chaffey 2002a). Trembling aspen (*Populus tremuloides* Michx.) is the most common *Populus* species in Canada, ranging over all of the Canadian provinces and most of the United States (Perala 1990; Dirr 1998). Trembling aspen is typically described as being a large tree, 15 to 25 meters tall and 6 to 9 meters wide at maturity (Dirr 1998). Young *P. tremuloides* crowns are conical in form, with excurrent branching whereby acutely oriented, orthotropic, branches follow a distinctly vertical central main stem (Millet et al. 1999; Dickmann et al. 2001). Terminal leader shoots added every year contribute to growth in height, while directing development in a vertical manner.

An unusual population of *Populus tremuloides* was discovered in the 1940's near Hafford, Saskatchewan, Canada. This clone presents an unique form that is quite different from that of wild-type trembling aspen trees, with individual stems having an overall twisted or crooked appearance (Remphrey & Pearn 2003). These trees have been known as the 'crooked aspen,' and appear to be the result of a genetic mutation. This interpretation is supported by experiments in which the crooked trait was retained through successive generations of vegetative propagation (Remphrey unpublished data). Early in the growing season (approximately May to early June) vigorous crooked aspen relay shoots begin developing in a relatively vertical manner, similar to that of wild-type shoots; however, with time, and continued extension growth, shoots

begin to lean slightly off vertical, followed by an abrupt bending of the shoot, usually downwards in relation to gravity. More dramatic shoot bending is observed with increased extension growth. The result is a crown composed of numerous crooked and twisted stems, with no central main stem, relatively limited vertical growth, and a more decurrent branching habit. Although the architecture of the crooked aspen is well understood (Remphrey & Pearn 2003), little is known about the mechanism that results in the bending of the shoots leading to the crooked form. Besides contributing to a better understanding of the crooked aspen, studying the mechanisms involved in shoot bending may provide additional insights into how woody plants orient and maintain their shoots in space.

Relatively few other instances of shoot bending have been reported in the literature. Certain pendulous species, including weeping mulberry (*Morus alba* var. *pendula*), and weeping Japanese cherry (*Prunus spachiana*) are explained as having long, thin shoots that cannot support their own weight and bend passively (Reches et al. 1974; Nakamura et al. 1995; Yoshida et al. 2000). In other species, delayed formation of adequate structural tissues (Zimmermann & Brown 1971; Alméras et al. 2004), or tissues involved in shoot reorientation (Yoshida et al. 2000) result in a range of shoot bending.

It is postulated that two processes might be involved in creating bent forms: *(i)* shoots are unable to support their own weight resulting in a biomechanical bending of the shoot, and/or *(ii)* shoot bending is caused by the formation of tissues that actively bend the shoot or cause differential extension

growth, resulting in shoot bending. The objectives of this project were to determine the extent to which passive and/or active mechanisms are involved in shoot bending in the crooked aspen by (1) characterizing the process and timing of bending, (2) assessing structural components of developing wild-type and crooked aspen leader shoot morphology, and (3) comparing anatomical properties of bending shoots with those of wild-type shoots.

# 2.0 LITERATURE REVIEW

## 2.1 Description of *Populus tremuloides*

*Populus tremuloides* Michx., commonly known as trembling aspen, is the most widely distributed tree in North America (Perala 1990; Dirr 1998). Its leaves flutter in the slightest breeze, thus the name *tremuloides*, from the Latin *tremulus* (trembling), and the Greek *öides* (resembling) (Perala 1990). It is a fast-growing, relatively short-lived tree that is quick to establish in disturbed sites and will invade unmanaged grasslands. As a young tree, trembling aspen commonly grows in pure stands (Farrar 1995). Trembling aspen is regarded as an early successional species able to dominate a site until replaced by more shade-tolerant, slower-growing broadleaf species and conifers (Perala 1990).

The native habitat of *Populus tremuloides* includes a wide range of climatic and soil conditions and can be found growing on a variety of soils ranging from moist, loamy sands to shallow rocky soils and heavy clays (Perala 1990; Dirr 1998). Trembling aspen is usually found associated with trees such as white and black spruce, balsam fir, paper birch, and balsam poplar. Commonly associated shrubs include beaked and American hazel, mountain maple, speckled alder, chokecherry, saskatoon, elder, highbush cranberry and redosier dogwood (Perala 1990).

Trembling aspen is a large tree, up to 25 m tall. Young trees have a narrow, pyramidal crown form, while older, stand-grown trees have rounded crowns atop long, narrow trunks devoid of branches due to self-pruning (Farrar

1995; Dirr 1998). Trembling aspen is a dioecious species, having male catkins and female seed catkins on different trees. Genetically diverse populations may arise from sexual reproduction; however, trembling aspen commonly forms large groups of genetically identical individuals, or clones. In aspen, such clones are formed when sucker shoots arise from the expanding root system of a single plant.

Trembling aspen is commonly used for wildlife habitat, and the wood can be used for pulp, particleboard, plywood, and lumber. Recently, researchers have promoted poplar (*Populus* spp.) as the model angiosperm tree (Mellerowicz et al. 2001; Chaffey 2002a). Dickmann et al. (2001) indicated that *Populus tremuloides* is the model species for "The Poplar Genome Sequencing Project." *Populus* spp. offer several advantages as model trees. They have high growth rates, are easy to propagate, have a relatively small genome, and have a large reservoir of genetic variability (Grunwald et al. 2001; Mellerowicz et al. 2001; Plomion et al. 2001; Chaffey 2002a).

## 2.2 Growth and Form in Woody Plants

### 2.2.1 Introduction

A woody plant can be defined as one that consists of a significant amount of xylem tissue providing both structural support and transport channels for water and nutrients (Mellerowicz et al. 2001). Woody plants increase in size and complexity year after year, adding new shoots and reinforcing existing stems. The process of both structural and functional development of an organism from its birth to its death is termed ontogeny (Smirnova et al. 1999). With numerous

different shapes and sizes of woody plants, attempts have been made to arrange species into two smaller groups, trees and shrubs. Guedes (1982) defined a shrub as a woody perennial made up of several erect stems inserted on a stock at or near the ground. A tree can be defined as a perennial woody plant with an erect elongated stock maintaining a myriad of perennial branches that form the crown.

Some of the most complex structural patterns in plants are found in trees (Fisher 1992). Trees develop below and above ground as a system of axes which are grouped in three parts: the crown, the trunk, and the root system (Edelin & Atger 1994). Each organ system is integral to the growth and development of the entire plant. The roots are large and woody and anchor the tree, while obtaining water and nutrients essential for plant growth. The trunk maintains a pathway for water and nutrient travel between the roots and the crown, while providing a structural foundation for the crown. The crown consists of a complex branching system supporting numerous leaves that function in photosynthesis, providing carbohydrates and energy for further plant growth and development. Structural botanists examine principles of organization, pattern, and form in an attempt to classify explain plant diversity (Fisher 1992).

### 2.2.2 Crown Structure and Form

#### 2.2.2.1 Branch Organization and Crown Form

Trees are the largest living organisms on earth. Because of their continued growth in height and breadth, trees have been the subject of many morphogenetic studies (Burk et al. 1983; Remphrey & Powell 1984; Ceulemans

et al. 1990; Davidson & Remphrey 1990; Prusinkiewicz et al. 1994; Thiébaut et al. 1998; Wilson 1998; Millet et al. 1999). Differences in the dimensions and the forms of tree crowns can be attributed to differences in rates of height growth, and to factors influencing: *(i)* the numbers and relative lengths of branches, *(ii)* the orientation of branch growth, and *(iii)* changes in growth and development with time (Jankiewicz & Stecki 1976).

Primary branches are often arranged to provide a stable distribution of branch weight and expose maximum leaf area for optimal photosynthetic rates (Burk et al. 1983). Thus, there are often progressive changes in branch angles and position within individual tree crowns, from more or less upright to acutely oriented (orthotropic) branches in the upper crown, to horizontally oriented (plagiotropic) branches near mid-crown, to somewhat drooping branches at the base of the crown (Zimmermann & Brown 1971).

#### 2.2.2.2 Apical Control and Crown Form

Differential elongation of buds and branches within a crown may be the result of apical control. Apical control in tree crowns can be defined as the physiological process whereby a leading shoot or apical bud inhibits the growth of subsequent lateral shoots and buds (Zimmermann & Brown 1971; Remphrey & Powell 1984; Wilson 2000). Tree species with strong apical control display a dominant main stem that outgrows the lateral branches beneath, giving rise to a cone-shaped and clearly defined central bole (Brown et al. 1967). This pattern of branching at the whole tree level is termed excurrent, and is common in most conifers and some angiosperms (Remphrey & Powell 1984). Young trembling

aspen trees have been described as having an excurrent crown structure with a distinct central main stem and orthotropic branches (Millet et al. 1999; Dickmann et al. 2001; Remphrey & Pearn 2003). In contrast, decurrent branching occurs in species with weak apical control, whereby lateral branches may outgrow the central stem giving a crown shape with no distinct leader. A number of hardwood species native to parts of Canada, including oak (*Quercus* spp.), elm (*Ulmus* spp.), and maple (*Acer* spp.) exhibit a decurrent growth habit (Brown et al. 1967; Millet et al. 1999). In decurrent species, branch angles are usually more acute than in excurrent forms (Zimmermann & Brown 1971; Takahashi et al. 2001).

Apical control influences bud development along parent shoots (Zimmermann & Brown 1971; Remphrey & Powell 1984; Wilson 2000; Remphrey et al. 2002). Champagnat (1978) observed that certain species have shoots with lateral buds that give rise to more vigorous shoots near the shoot tip than those at the base. This pattern of development is known as acrotony (Champagnat 1978; Remphrey & Pearn 2003). In contrast, some species display a developmental pattern of basitony whereby the longest, most vigorous shoots arise from basal regions of the parent stem. Basitonic species are said to have weak apical control while species with acrotonic development show strong apical control. Basal shoots within stems displaying acrotonic development are believed to be inhibited by those growing distal to them (Maini 1966; Jankiewicz & Stecki 1976; Remphrey & Powell 1984; Wilson & Gartner 2002). It has been observed that apical control may be influenced by gravity in that terminal buds are recognized as those furthest from the gravitational stimulus (Jankiewicz et al.

1967; Jankiewicz & Stecki 1976; Cline 1991; Wilson 1997). Wild-type trembling aspen displays acrotonic growth (Jankiewicz & Stecki 1976; Isebrands & Nelson 1982). In contrast, the crooked aspen has been described as having a pattern known as mesotony whereby the most vigorous shoots arose from more central regions of the parent stem (Remphrey & Pearn 2003).

In examining bud development, Cline (1991) used the term apical dominance to refer to the control of the amount of lateral growth in addition to the release from inhibition. It has been suggested that apically produced hormones as well as nutrient movement to the terminal bud might have a role in promoting apical dominance (Cline 1991). Auxin is thought to be the most important hormone involved in apical control in woody plants. Auxin, produced in the shoot apical region, moves basipetally down the stem and into the lateral buds where it appears to suppress lateral bud outgrowth through its interaction with receptors in the plant cell (Wareing & Nasr 1961; Zimmermann & Brown 1971; Leakey & Longman 1986; Cline 1991; Salisbury & Ross 1992; Raven et al. 1999). Also, developing buds require nutrients for growth. It has been indicated that the preferential movement of nutrients to the terminal bud (a strong metabolic sink) could be involved in apical dominance, creating an uneven competition between buds for nutrients (Cline 1991; Wilson 2000), resulting in differential extension growth. In *Malus* species, known to exhibit apical dominance, Borkowska (1975) demonstrated that the uppermost buds attracted $^{14}C$-labelled carbohydrate reserves more readily than basal buds.

### 2.2.2.3 Architectural Models of Tree Form

Hallé et al. (1978) defined tree architecture as the "morphological expression of the genetic blueprint (i.e. program) of a tree at any one time." A tree's architecture is not only influenced by its genetics, but also by environmental factors. Successive observations of a tree's architecture during ontogeny is referred to as its architectural model (Hallé et al. 1978). Fundamental characters which establish tree shape, including pattern of branching, growth direction of axes, and location of flowers, are used to develop individual architectural models (Edelin & Atger 1994).

Hallé et al. (1978) have classified trees into several qualitative models according to their architectural patterns. These models vary in complexity, from relatively simple, monoaxial, unbranched trees, to more complex, polyaxial, branched trees. Within the polyaxial trees, Hallé et al. (1978) distinguished between those with morphologically equivalent and orthotropic axes, those with vegetative axes that are differentiated into trunk and branch, and those with mixed axes, that is, the main shoots or branches are part vertical and part horizontal. *Populus tremuloides* is best described by Rauh's model (Millet et al. 1999; Remphrey & Pearn 2003), characterized as having a monopodial trunk (an axis produced by a single apical meristem) producing levels of morphogenetically identical branches (Hallé et al. 1978). Rauh's model is common in tree species of the temperate regions.

Trees with mixed axes have been classified into three models (Hallé et al. 1978). In these models, the geometric and physiological orientation of the axis

changes in relation to the activity of a single meristem (Hallé et al. 1978). In Mangenot's model growth is initially more or less orthotropic followed abruptly by a plagiotropic phase, yielding shoots with relatively upright basal portions and horizontal to pendulous distal portions (Hallé et al. 1978). Champagnat's model differs in that the axis is initially orthotropic and then the distal portion bends secondarily under its own weight. Finally, Troll's model differs from both Mangenot's and Champagnat's in that axes are initially plagiotropic, followed developmentally by secondary erection of the basal portion of the axis (Hallé et al. 1978). The mechanisms and relative timing of the development of mixed axes in each of these models further distinguishes them. The change may be primary (progressively bending as the shoot develops), as in Mangenot's model, or secondary changes (following primary growth) may contribute to shoot reorientation as in Champagnat and Troll's models. Mangenot and Troll's models involve the active bending of shoots, while Champagnat's model describes passive bending due to the inability to support its own weight. Remphrey and Pearn (2003) described the crooked aspen as having an architecture that resembles Champagnat's model but were not able to confirm that the shoots bent under their own weight.

## 2.3 Stem Orientation in Woody Plants

### 2.3.1 Introduction

Woody plants maintain the same position on earth from the time they set down their first root to their death. However, trees do have the ability to respond to a wide range of changes in their external environment, and make adjustments

in their form (Raven et al. 1999). Although some stem orientations observed in crowns are genetically predetermined, woody plants manipulate stem orientation through several active mechanisms. These mechanisms are based on the amount and distribution of wood produced by cambial activity, the stress level generated by the wood, and the amount and distribution of reaction wood (Wilson & Archer 1983; Yamamoto et al. 2002; Alméras et al. 2004). A stem is said to have reached its equilibrium position when it does not produce wood having any of these differential growth stresses (Wilson & Archer 1977; Wilson 2000).

### 2.3.2 Influences on Stem Orientation

#### 2.3.2.1 Tropic Responses of Woody Stems

Branches within a tree crown may be regulated in their direction of growth by bending or curving toward or away from an external stimulus such as light and gravity (Zimmermann & Brown 1971; Hangarter 1997). Such responses are referred to as tropisms (Myers et al. 1994; Raven et al. 1999). Phototropism is the directional bending of plant organs in response to the light stimulus (Hangarter 1997; Correll & Kiss 2001) to maximize their ability to obtain maximum photosynthetically active radiation. Gravity is one of the most important formative factors in plants because of its continuousness, uniform intensity, and constant direction (Zimmermann & Brown 1971). Gravity, therefore, provides an important cue for orienting plant growth (Barlow 1995; Hangarter 1997). The bending of an organ in response to gravity is referred to as gravitropism (Fukaki et al. 1998; Ranjeva et al. 1999; Correll & Kiss 2001). Generally, plant roots grow at some angle down toward the gravitational

stimulus, while stems usually grow up, at some angle away from the gravitational stimulus. However, trees maintain branches at various angles off vertical. Digby and Firn (1995) introduced the notion of the gravitropic set-point angle (GSA) defined as the angle with respect to gravity at which a branch shows no gravity-induced differential growth. When a branch is bent out of its GSA it can actively restore its original orientation (see section 2.3.4) (Yoshida et al. 2000; Yamamoto et al. 2002).

The orientation of some plant shoots can change with time, often bending down due to environmental stimuli and self-weight; however, with a process known as gravitropic sign-reversal (Digby & Firn 1995), the youngest, most recently formed internodes at the tip of the shoot have a negative gravitropic response and bend up (Myers et al. 1994; Fournier et al. 1994; Jirasek et al. 2000). Jourez and Avella-Shaw (2003) found that developing *Populus euramericana* shoot tips responded to artificial stem inclination after 2 hours, and returned to a vertical position after 4 hours. Crooked aspen shoot tips have been observed to bend upwards, showing negative gravitropism, while adjacent parts of the shoot bent down (Remphrey & Pearn 2003).

Correll and Kiss (2001) distinguished three similar events in both phototropism and gravitropism: *(i)* sensing of the light / gravity signal, *(ii)* transduction of the signal, and *(iii)* differential growth of organs. It is believed that blue light photoreceptors enable plant stems to sense light (Correll & Kiss 2001). Gravity perception in the root is generally associated with the sedimentation of amyloplasts located in cells of the root cap (Sack 1991; Sievers et al. 1996; Chen

et al. 1999). Gravity perception in the shoot is poorly understood. Signal transduction in tropic responses is also unclear, but Correll and Kiss (2001) indicated that calcium may be involved in both perception and signal transduction. Hangarter (1997) reported that the redistribution of certain plant hormones within stems is generally considered to be responsible for the differential growth related to gravitropism. Auxin is the plant hormone that is considered to be particularly important in cell elongation and thus is very important in woody plant development. It is widely known that lateral differences in auxin distribution play a major role in stem movement, including tropic bending (Wareing & Nasr 1961; Zimmermann & Brown 1971; Wilson & Archer 1977; Raven et al. 1999; Dickison 2000; Mellerowicz et al. 2001).

#### 2.3.2.2 Mechanical Influences on Stem Orientation

Other major influences on overall stem orientation include various mechanical forces coming from the plant's surrounding environment. Forest trees and shrubs are subjected to chronic bending from self-weight as well as traumatic bending from snow load, wind sway, and/or debris falling from the overstory (Zimmermann & Brown 1971; Wilson 1997; Zipse et al. 1998). Mechanically bent stems may respond by actively manipulating stem orientation (see section 2.3.3 and 2.3.4), restoring the orientation of the stem. Otherwise, the plant may follow a "throwaway" strategy, making a new leader stem and leaving the old, bent stem in place (Wilson 1997). In fruit trees, excessive fruit loading influences stem orientation and subsequent shoot development to

compensate for differential growth strains (Kervella et al. 1994; Alméras et al. 2002; Alméras et al. 2004).

### 2.3.3 Passive Mechanisms for Stem Bending: Strength and Elasticity

#### 2.3.3.1 Wood and Stem Strength

Essential to woody plant growth is the development of secondary tissues, or wood. Secondary tissue gives mechanical support while providing a bi-directional pathway for long-distance transport of water and nutrients (Zimmermann & Brown 1971; Kervella et al. 1994). Wood is functionally the most important support tissue in trees and is devoted to maintaining the stability of the whole organism (Edelin & Atger 1994). In addition, Niklas (1999) provided evidence that bark (all tissues external to the vascular cambium) acts as a significant stiffening agent in young woody stems. From an engineering standpoint the location of bark is ideally suited for mechanical support in stems with relatively little wood development.

The massive size attained by woody plants is made possible by correlations between extension and radial growth (Zimmermann & Brown 1971). It is generally agreed that there is a linear relationship between stem length and its diameter (Burk et al. 1983; Cannell et al. 1988). As a stem increases in length and therefore weight, it must increase in diameter (Cannell et al. 1988), thereby increasing stem strength. In examining branches of several deciduous tree species, Niklas (1999) found that the radial thickness of the wood increased exponentially with segment age, while the bark thickness increased as a linear function of age. In observing stems at an angle off vertical, Cannell et al. (1988)

reported that branch biomass increased steeply, in a non-linear fashion, with an increase in stem length. Therefore, long branches have significant support costs. Osler et al. (1996) reported a rapid increase in stem radial growth following an external bending stress. Bent seedlings also developed a much sharper taper along their stems as a mechanism to tolerate stem bending (Leiser & Kemper 1973; Fournier et al. 1994; Osler et al. 1996). The same follows for trees that sway in the wind, with trunks developing increased taper towards the base (Larson 1965; Zimmermann & Brown 1971). Remphrey and Pearn (2003) found a close relationship between stem length and basal diameter, but indicated that as the stems became longer, wild-type aspen stems had an increasingly smaller diameter than comparable-length crooked aspen stems.

Organisms with decreased stem strength tend to maintain a variety of stems bent at various angles from the vertical (Zimmermann & Brown 1971). Zimmerman and Brown (1971) offered a hypothesis that the timing between extension growth and adequately strengthened tissues for mechanical support may result in the development of drooping or prostrate forms. Yoshida et al. (2000) confirmed that branches of weeping Japanese cherry are pendulous because the rate of shoot elongation is greater than the thickening rate, so branches cannot support their own weight. In an analysis of stem form in different apricot varieties, Alméras et al. (2004) found that the more pendulous species had narrow shoots at the time of fruit maturation and therefore bent passively under the load. Remphrey and Pearn (2003) hypothesized that the mutant crooked aspen may be unable to retain orthotropic growth because of

lack of strength. Unfortunately, there has been relatively little research on the relationship between weeping forms and the lack of stem strength.

#### 2.3.3.2 Young's Modulus

Young's modulus (*E*) measures the effective rigidity or resistance to deflection of woody plant stems (Cannell & Morgan 1987). Young's modulus is known to increase, stems becoming more rigid, with increased specific gravity (Cannell & Morgan 1987; Cannell et al. 1988). Therefore, the mass of wood within a stem is closely coupled with its ability to provide mechanical support. Cannell et al. (1988) found that as *E* decreased (towards greater flexibility), the diameter required for self-support increased. Also, Young's modulus decreases with an increase in stem water content (Cannell & Morgan 1987). Speck (1994) found that in self-supporting woody plants *E* increases during ontogeny, while in non-self-supporting plants *E* decreases during ontogeny. The Young's modulus of *Acer saccharum*, *Fraxinus americana*, and *Quercus robur* branches was shown to increase with age (Niklas 1999). Isnard et al. (2003) found that the structural Young's modulus of developing *Clematis flammula*, a woody climbing and non-self-supporting plant, decreased with increasing stem development.

### 2.3.4 Active Mechanisms for Stem Orientation and Reorientation

#### 2.3.4.1 Differential Cambial Activity and Reaction Wood

In creating a specific form, trees must orient and sometimes reorient lateral branches and the leader axis (Fisher & Stevenson 1981). Mechanically, trees are able to adapt and respond to their immediate environment by controlling the development of wood, its location, and its structure (Edelin & Atger

1994). Woody plants are able to actively manipulate stem orientation by creating growth stresses within their woody tissue. The development of differential growth stresses in wood requires an increase in cambial activity and wood production (Wilson 1998). Therefore, an increase in stem diameter should be observed in reorienting stems. However, Alméras et al. (2004) indicated that the shape of a stem depends not only on the amount of diameter growth, but also on the tissues found within the stem. Hejnowicz (1997) introduced the term tree growth stresses as the process by which new cells, added by the vascular cambium, shrink longitudinally and expand in the transverse direction during cell wall maturation. An asymmetrical arrangement of these unique cells within woody stem tissue produces internal bending moments (Wilson & Archer 1977; Fournier et al. 1994; Yamamoto et al. 2002; Alméras et al. 2004). These differential growth stresses can function to bend branches or stems to maintain a particular architecture, or to replace injured or missing branches (Niklas 1992; Fournier et al. 1994; Wilson 1998). Lateral stems, held at an angle off vertical must generate an upward bending moment from differential growth stresses in new wood to overcome the downward bending force from gravity and other mechanical stresses (Hejnowicz 1997; Wilson 2000).

One mechanism in which the stem is able to actively bend is through the production of reaction wood (RW). Reaction wood, including both tension wood (TW) in angiosperms and compression wood (CW) in gymnosperms, is formed in secondary xylem tissue (wood). Tension wood is typically found on the upper side of leaning stems while compression wood is found on the lower side of

leaning stems (Hejnowicz 1967; Wilson & Archer 1977; Niklas 1992; Wilson 1997; Jourez & Avella-Shaw 2003). The mechanical action of reaction wood results from the contraction (in the case of TW) or expansion (in the case of CW) of stem tissues, thus developing growth strains. These growth stresses produce internal bending moments which can cause changes in curvature and reorientation of the stem (Wilson & Archer 1977). Poplar species frequently produce tension wood (Hejnowicz 1967; Mia 1968; Wilson & Archer 1977; Mellerowicz et al. 2001; Hellgren et al. 2004), and Jourez et al. (2001) found that *Populus euramericana* is very sensitive to stimuli responsible for TW formation.

Tree branches will form tension wood on the top if bent down and on the bottom if bent up so that the bending response returns them to a position where no tension wood is formed (Hejnowicz 1967; Wilson 1997), similar to the concept of the GSA (see section 2.3.2.1) (Digby & Firn 1995). However, tension wood has been observed on upper and lower sides of young (one- and two-year-old) inclined shoots (Fisher & Stevenson 1981; Jourez & Avella-Shaw 2003). Tension wood was found in the oldest (first formed) secondary xylem on the lower side of the shoot, and in xylem tissue formed later that same year on the upper side of the shoot (Fisher & Stevenson 1981; Jourez & Avella-Shaw 2003). This sequence of development would predict that young branches would be initially pulled down to a desired angle and subsequently held there, which correlates with observed increases in branch angles (Fisher & Stevenson 1981). In experimentally inclined *Pinus* shoots, Fournier et al. (1994) found evidence of compression wood on both the upper and lower sides of reorienting shoots,

acting first to bend the shoot up vertically, and then to hold it in that position, preventing the shoot from bending over the vertical. Yoshida et al. (2000) observed tension wood formation on the upper side of the pendulous branches of weeping Japanese cherry (*Prunus spachiana* Kitamura f. *spachiana* cv. *Plenarosea*). Tension wood was absent from shoots suspended at near-vertical angles. They concluded that branches weep because of their own weight and form tension wood in an attempt to reorient themselves toward the vertical (Yoshida et al. 2000). No evidence was found to indicate that original bending downwards was caused by tension wood formation on the lower side of shoots. Niklas (1992) indicated that the intensity of the tensile stresses developing within each branch is highest in the region of bending. Thus, there is a strong correlation between the location of TW and a change in the orientation of a stem during normal growth (Wilson & Archer 1977; Fisher & Stevenson 1981).

Inclined or bent young current-year shoots with a large proportion of soft tissues to secondary xylem are also capable of generating reaction wood to reorient themselves (Hejnowicz 1967; Fisher & Stevenson 1981). Jourez and Avella-Shaw (2003) found evidence of TW formation in young, developing, current-year *Populus euramericana* shoots that had been experimentally inclined for only 48 hours. However, Hejnowicz (1967) reported that while tension wood was present in the wood of current-year shoots of *Populus tremula* during the growing season (in August), no upward bending was observed until the leaves had fallen at the end of the season (in October). Alméras et al. (2004) indicated that while tension wood was present and functional in two-year-old apricot stems,

it could have a greater role in older stems. Hejnowicz (1967, 1997) presented a hypothesis that differences in water content may have resulted in differential shrinkage of tension wood compared to normal wood. A decrease in water content during maturation, after the leaves had fallen, might have resulted in the longitudinal shrinkage of tension wood fibres during drying. Except for these few studies, there has been little research on reaction wood formation in young, current-year shoots.

Cell differentiation and lignification are influenced by growth regulating hormones (Dickison 2000; Mellerowicz et al. 2001; Ko et al. 2004). Hormones such as auxin and gibberellins stimulate plant growth by promoting the extensibility of cell walls (Raven et al. 1999). Auxin is also known as a major signal involved in stimulating the development of secondary vascular tissues (Ko et al. 2004). Reaction wood formation is thought to be influenced by auxin in that compression wood forms under high auxin concentration and tension wood forms under low auxin concentration (Wilson & Archer 1977; Plomion et al. 2001; Hellgren et al. 2004). Wilson and Archer (1983) proposed that TW forms under low auxin concentrations brought about by lateral transport, reducing the auxin concentration on the upper side of leaning stems or branches. Wareing and Nasr (1961) confirmed that under the influence of gravity, auxin tends to accumulate on the lower side of horizontal organs, thus resulting in TW formation on the upper side. Applied auxins and auxin transport inhibitors have been found to regulate reaction wood formation in that auxin applied to the upper side of

branches can arrest the formation of tension wood (Wilson & Archer 1977; Hellgren et al. 2004).

No studies have confirmed the role of auxin in the development of weeping shoots in woody plants. Reches et al. (1974) found no lateral differences in auxin distribution in developing shoots of weeping mulberry. They hypothesized that bending was the consequence of decreased sensitivity to auxin levels, and therefore decreased extension growth, on the lower side of the stem, leading to stem bending. Remphrey and Pearn (2003) suggested that one possibility for the bending seen in the shoots of the crooked aspen may be related to increased auxin transport to one side of the shoot.

### 2.3.5 Stem Orientation and Gravimorphism

The position of the stem in relation to gravity has a marked effect on its subsequent growth and development. The development of branches on young trees depends on their positions relative to gravity (Jankiewicz & Stecki 1976; Wilson 2000). Wareing and Nasr (1961) introduced the term gravimorphism to describe the effect of gravity on the subsequent development of stems oriented at some angle off vertical. In all species studied, they found that total annual extension growth was noticeably less in horizontally grown trees compared to the same species grown vertically (Wareing & Nasr 1961). In their experiments, stems oriented horizontally showed decreased apical control compared to those held vertically (Wareing & Nasr 1961; Little & Lavigne 2002). Jankiewicz and Stecki (1976) confirmed that placing poplar trees in a horizontal position at bud-burst decreased its acrotonic tendency, and eventually induced a completely

basitonic habit. There is also a tendency for daughter shoots to grow out on the upper side of horizontal or arched parent shoots (Wareing & Nasr 1961; Borkowska & Jankiewicz 1972; Little & Lavigne 2002). Jankiewicz et al. (1967) found that buds on the lower side of horizontally oriented hybrid poplar shoots either did not develop or had relatively little growth, while shoots on the upper side grew vigorously. In observing the crooked aspen, Remphrey and Pearn (2003) found that daughter shoots arising from the upper surface were longer than those arising from the lower side, indicating that the phenomena of gravimorphism was expressed. Wareing and Nasr (1961) suggested that nutrients are diverted to the highest upwardly directed meristem, and therefore, the most vigorous development of lateral buds occurs at the nearest point to the roots at which the stem is diverted from the vertical position.

## 2.4 Structural Stem Anatomy

### 2.4.1 Introduction

Knowledge of the anatomy of any plant system is fundamental to gain an understanding of its development (Chaffey 2002a). This section will focus on the anatomy of woody angiosperms exhibiting secondary growth, with specific reference to *Populus* where possible. Chaffey (2002a) indicated that the anatomy of *Populus* has been studied and is a good representative of hardwood tree anatomy.

### 2.4.2 Primary Shoot Structure

The shoot apical meristem is responsible for the continued growth of the stem. In the primary stages of shoot development new cells are variously

dividing, enlarging, and differentiating into respective tissues (Bowes 1996; Dickison 2000). Most young shoots grow rapidly, and the elongating regions are often long and thin. Such primary shoots require specialized structural tissues that allow for extension growth, while providing stability to the shoot (Mauseth 1988). Relatively little research exists that examines the structural role of primary tissues in young woody shoots.

Three major tissue systems are found in the primary shoot structure of all plants: the dermal tissue system, the ground tissue system, and the vascular tissue system. The dermal tissue system includes the epidermis, a single layer of specialized cells on the outside of the shoot. The epidermis functions as a protective boundary layer between the environment and internal plant tissues (Mauseth 1988; Dickison 2000). The ground tissue system includes the cortex, the region between the epidermis and the outermost cells of the vascular cylinder, and the pith, interior to the vascular cylinder. Cortex tissue is made up of parenchyma and collenchyma cell types. Parenchyma is a broad classification for cells having only primary cell walls (Mauseth 1988; Raven et al. 1999). Parenchyma cells have various functions including photosynthesis, short distance transport, and storage. Collenchyma is considered to be the mechanical tissue of young, growing organs (Mauseth 1988; Dickison 2000). Collenchyma cells have irregularly thickened cell walls providing strength while maintaining a plastic nature. Plasticity allows cells to be stretched or pushed into a new shape, retaining the new shape when the tension or pressure is released.

Collenchyma cells are typically found in peripheral regions of developing organs, often forming a layer directly beneath the epidermis (Bowes 1996).

In the primary stages of shoot development the vascular tissues of woody angiosperms, including *Populus* species, are arranged as discrete vascular bundles, forming a cylinder around the central pith. *Populus* species exhibit the most common type of vascular bundle, collateral bundles, with primary xylem to the inside and primary phloem to the outside (Mauseth 1988). The stage of primary development is relatively short-lived in *Populus*, as secondary growth commences early in the growing season.

Angiosperm xylem tissue is made up of various specialized cell types including vessel elements, tracheids, several types of fibres, and parenchyma cells (Mauseth 1988; Mellerowicz et al. 2001). Early in shoot development, primary xylem functions mainly in the conduction of large quantities of both water and certain organic and inorganic solutes (Dickison 2000). In angiosperms, vessel elements and tracheids are the two principal conductive cell types of the xylem, but fibres and parenchyma cells are also capable of limited active transport (Raven et al. 1999; Dickison 2000). Vessel elements have a relatively wide central cavity, or lumen, and are lined up end-to-end forming an extended hollow cylinder, or vessel, that enhances the rapid movement of water (Mellerowicz et al. 2001). Xylem parenchyma may function in water and/or nutrient storage. With the onset of secondary development and cell wall thickening, fibres may provide mechanical support for the whole plant or its parts

(Mauseth 1988). The structural role of secondary xylem tissue is discussed in section 2.4.3.

Primary phloem tissue includes conductive cells such as sieve tube elements and companion cells. These tissues function in the transportation of organic nutrients, especially the sugars produced in photosynthesis, from one part of the plant to another (Mauseth 1988). Phloem fibres are the last of the primary phloem to form (Raven et al. 1999). Primary phloem fibres are made up of a specialized cell type, sclerenchyma, with thickened secondary walls that are lignified at maturity (Den Outer 1993; Chaffey et al. 2000; Quilhó et al. 2000). Sclerenchyma is another significant form of mechanical tissue that occurs in plants; however, unlike collenchyma tissue, which is considered as plastic, sclerenchyma cell walls are considered to be elastic in nature (Mauseth 1988; Quilhó et al. 2000). An elastic cell wall can be deformed by either tension or pressure (just like a plastic wall) but resumes its original size and shape when the deforming force is removed (Mauseth 1988). Sclerenchyma tissue is therefore an ideal tissue for reinforcing a shoot that has developed its mature shape and size. If a mature stem is reinforced by plastic tissues, then external forces such as wind, snow-load, or fruit weight would cause deformations that would be permanent (Mauseth 1988). Because of the elasticity of sclerenchyma, once the wind stops or the snow melts or the fruit falls, the stem returns to its proper shape and orientation (Niklas 1999). Phloem fibres are typically distributed singly or in a small tangential row near the perimeter of the stem, as in *Populus* species (Den Outer 1993; Niklas 1999; Dickison 2000). It is believed

that auxin plays a role in primary phloem fibre lignification (Mellerowicz et al. 2001). These thick-walled fibres are the only primary phloem that remains intact following secondary expansion of the plant body.

### 2.4.3 Secondary Stem Structure

In herbaceous plants, organs are made up of entirely primary tissues, but woody species are able to initiate a new phase of development called secondary growth (Mauseth 1988). In contrast to the primary growth produced by shoot apical meristems, secondary growth results from the activity of lateral meristems: the vascular cambium and the cork cambium (Dickison 2000; Chaffey 2002a; Helariutta & Bhalerao 2003). Secondary (lateral) meristem activity is responsible for an increase in the lateral growth, or girth, of the organ. For the purpose of this section only the vascular cambium and its products will be discussed as the vascular cambium is the major secondary meristem responsible for producing tissues for mechanical support.

#### 2.4.3.1 The Vascular Cambium

The onset of cambial activity marks the end of the primary phase of shoot development and begins secondary development. Through periclinal divisions of cambial initials, secondary xylem develops inwards or centripetally, and secondary phloem develops outwards or centrifugally (Niklas 1999; Chaffey 2002a; Helariutta & Bhalerao 2003). The activity of the vascular cambium produces an uninterrupted cylinder of secondary vascular tissue (Chaffey 1999; Dickison 2000). The amount of secondary phloem is usually much less than the amount of secondary xylem produced (Niklas 1999). In all woody plants the

vascular cambium functions cyclically: it is active during favourable times of the season and quiescent when conditions are too cold, too hot, or too dry (Mauseth 1988). This periodicity results in annual increments of secondary growth, or annual rings.

#### 2.4.3.2 Secondary Xylem Tissue: Wood

Woody plants develop large frames, and therefore require tissues that are functional in support as well as in long-distance conduction of water and nutrients. Secondary xylem has evolved to fulfill both of those needs (Mauseth 1988; Mellerowicz et al. 2001). Secondary xylem, or wood, develops by a succession of four major steps, including cell division, cell expansion, cell wall thickening, and programmed cell death (Plomion et al. 2001).

Early in the growing season, the xylem is primarily functioning in the rapid, large-volume conduction of water, whereas in the summer it functions more in mechanical support with relatively less water transport required (Mauseth 1988). Consequently, in the spring, xylem elements are noticeably wider than the narrower, thicker-walled xylem fibres in the summer (Dickison 2000). *Populus* species are known to have diffuse porous woods, with vessel elements of similar diameter distributed evenly throughout all xylem tissue (Jourez et al. 2001), in contrast with ring porous woods having numerous large vessels and few fibres formed in the spring, and mostly fibres formed in the summer (Mauseth 1988).

The mechanical support and rigidity of the secondary xylem is the result of the deposition of secondary cell walls. Once expansion is completed, the formation of the secondary cell wall begins with the biosynthesis and assembly of

three major compounds: polysaccharides (cellulose and hemicelluloses), lignins, and cell wall proteins (Okuyama et al. 1994; Plomion et al. 2001; Junghans et al. 2004). Cellulose microfibrils are oriented randomly in the primary wall but are highly organized in the secondary wall. Lignin is deposited within the polysaccharide matrix giving rigidity and cohesiveness to the tissue, providing a hydrophobic surface ideal for the transport of water (Mellerowicz et al. 2001; Donaldson 2001; Plomion et al. 2001). Xylem fibres, unique to most angiosperms, are one type of secondary cell that provides considerable support. These fibres are elongated, tapered, sclerenchyma cells with thick, lignified secondary walls (Dickison 2000). Xylem fibres are similar to phloem fibres, providing elastic strength and support to the stem (Mauseth 1988). Dickison (2000) indicates that some cells, including those with strengthening and supporting functions, begin adding wall material inside the primary wall during cell expansion, before the cell has reached its final size. The amount of lignin and cellulose laid down in the secondary wall is influenced by the genetics of the organism as well as by abiotic factors such as mechanical stress (Plomion et al. 2001). Woods with many thick-walled cells are stronger, heavier, and stiffer than wood with thin-walled cells (Raven et al. 1999; Dickison 2000).

#### 2.4.3.3 Secondary Phloem Tissue

Similar to the secondary xylem, the secondary phloem arises from the vascular cambium and possesses both axial and radially oriented tissue systems (Mauseth 1988; Dickison 2000). The radial tissue system is very similar and often continuous with that of the secondary xylem. The axial tissue system

includes secondary sieve tube elements, companion cells, phloem parenchyma, and secondary phloem fibres (Dickison 2000). Like the primary phloem, one of the main functions of secondary phloem elements is in long distance transport (Zimmermann & Brown 1971). Similar to the primary phloem, the sclerenchyma of secondary phloem fibres can be an important source of support in younger twigs, particularly due to its location in the periphery of the stem, which is ideal from a mechanical standpoint (Mauseth 1988; Den Outer 1993; Niklas 1999).

### 2.4.4 Tension Wood Anatomy

In dicotyledons, greater cambial activity towards the upper side of leaning stems results in stem eccentricity. In some instances this eccentric wood shows qualitative differences from normal wood. These tissues are commonly arranged with tension wood (TW) forming on the upper side of the stem, and opposite wood (OW) on the lower side. Jourez et al. (2001) found that stimuli involved in tension wood formation affects both phases of wood development: differentiation and maturation. During differentiation, the stimulus affects the numbers of cells (vessel elements, fibres, or ray parenchyma). During maturation, the stimulus affects dimensions of cells (length and wall thickness).

Anatomical evidence for tension wood is most dramatically observed in xylem fibres on the upper side of leaning stems (section 2.3.4.1). In the initial stages of tension wood formation, xylem fibres appear normal until an inner gelatinous layer (G-layer) within the secondary walls is produced (Zobel & Van Buijtenen 1989; Niklas 1992; Krishnamurthy et al. 1997; Jourez et al. 2003; Alméras et al. 2004). Such gelatinous fibres (G-fibres) contain one or more

lignified outer layers in the secondary wall and a substantial, gelatinous inner layer (Boyd 1977). The G-layer lacks lignin and is characterized as being mainly cellulosic with low microfibril angles (Mia 1968; Wilson & Archer 1977; Fisher & Stevenson 1981; Jourez et al. 2001; Carlquist 2001). The cellulose microfibrils are laid down as extended, longitudinally oriented springs and thus exert a tensile force tending to right or stabilize a stem (Okuyama et al. 1994; Bamber 2001). Low amounts of lignin facilitate the contraction of cellulose microfibrils, thus maximizing tensile stress. There is a close correlation between sites of G-fibres and the direction of axis movement (Fisher & Stevenson 1981; Okuyama et al. 1994).

Other anatomical anomalies in the secondary plant body are known to be associated with tension wood formation. An increase in gelatinous fibre formation is typically accompanied by a significant reduction in size and number of vessel elements produced in the xylem tissue of tension wood (Wilson & Archer 1977; Fisher & Stevenson 1981; Zobel & Van Buijtenen 1989; Niklas 1992; Krishnamurthy et al. 1997). Jourez et al. (2001) found that vessel frequency in tension wood was 33% lower than in opposite wood. Also, ray parenchyma in tension wood may differ from that of normal wood. With increased stem asymmetry, secondary phloem rays become dilated tangentially as a means of accommodating increased growth in diameter (Fisher & Stevenson 1981).

Boyd (1977) and Krishnamurthy et al. (1997) indicated that where tension wood cells occur in xylem, many fibres in adjacent phloem frequently contain an

inner, thick, unlignified, cellulosic layer similar to that of G-fibres in the xylem. In examining various species of Leguminosae, Krishnamurthy et al. (1997) found that in some taxa, G-layers were present in secondary phloem fibres even before tension wood and the accompanying G-fibres could be located. This provides evidence that phloem tissue is influenced by external stimuli even prior to its influence on secondary xylem.

# 3.0 MATERIALS AND METHODS

## 3.1 Plant Material

### 3.1.1 Study Sites

For this study, specimens of crooked *Populus tremuloides* trees growing at the University of Manitoba research arboretum were selected. These trees were propagated vegetatively from one identical clone found near Hafford, Saskatchewan, and ranged in age from 6 to 11 years old. Ten-year-old wild-type trembling aspen trees were selected randomly from a uniform bluff located at King's Park, just south of the University of Manitoba. The selected trees were suckers (ramets) that probably developed from the same clone (genet); however, it was not possible to verify this in this study.

### 3.1.2 Shoot Material

For the purpose of this study a shoot is defined as an annual increment of growth arising from an overwintering bud and ending in a terminal bud (Wilson 1998; Remphrey et al. 2002). Wild-type and crooked aspen shoots exhibit two morphologically distinct shoot types commonly referred to as long shoots and short shoots (Zimmermann & Brown 1971). Hallé et al. (1978) indicated that long shoots produce growth in length and their proliferation adds to the overall structure of the tree, while short shoots usually have a specialized function such as localized sites for reproductive structures and photosynthetic area.

The purpose of this study was to compare shoot development as it relates to the overall architecture of each tree type. Therefore, only long shoots

considered to be playing a significant role in expanding the crown were sampled. These shoots were called leader shoots. Leader shoots within wild-type trembling aspen crowns typically arise from the terminal bud of the central stem, contributing to the overall acrotonic form (Jankiewicz & Stecki 1976; Isebrands & Nelson 1982) (Figure 1a). Remphrey and Pearn (2003) found that the vigorous crooked aspen shoots typically arose from the upper side of the central region of the parent stem (Figure 1b). Because of their non-specific origin crooked aspen leader shoots (or relay shoots) are more difficult to locate early in development. Therefore, those developing into short shoots and non-vigorous long shoots were eliminated once they were identified. Non-vigorous long shoots were defined as shoots that had not attained a length of 40 cm as of July 19, 2004. Subsequently, various characteristics of wild-type and crooked aspen shoots were compared.

### 3.2 Measurement and Modeling of Crooked Aspen Shoot Bending

Measurements of crooked aspen shoot development were made at several dates from the bud stage to the end of the growing season. Shoot reconstruction models were created to capture the morphology of shoot bending.

#### 3.2.1 Shoot Selection

Before shoots had emerged from the buds, putative leader-shoot-producing buds were selected and recorded within 6 different crooked aspen crowns. Because the digitizer relay (see section 3.2.2) was located in a fixed position on each measurement date it was necessary to select shoots in close proximity to each relay site. Thus, the field area was divided into four

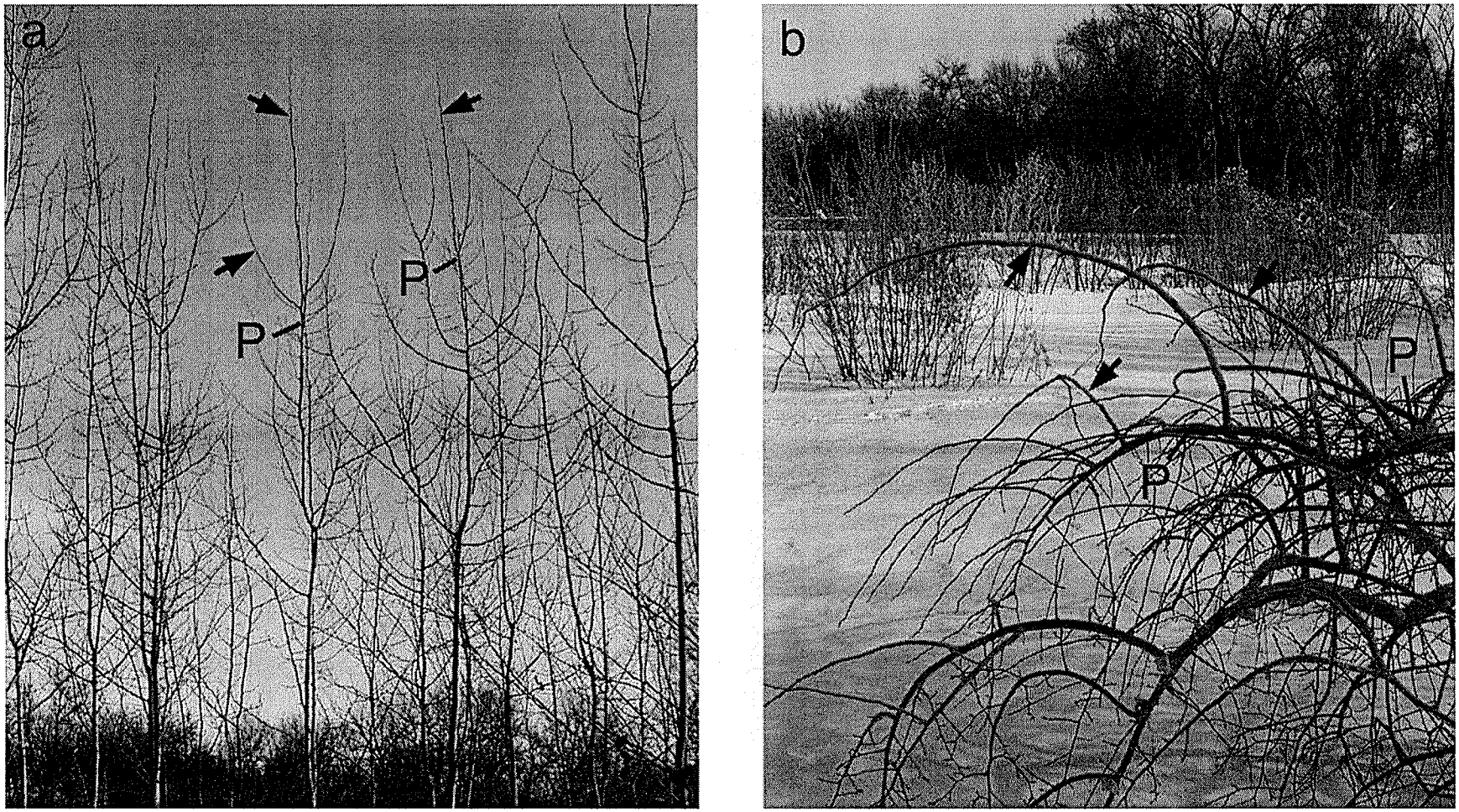



**Figure 1.** Wild-type *Populus tremuloides* (a) and crooked *P. tremuloides* (b) branching patterns. Wild-type shoots display acrotonic development with the longest lateral shoots (arrows) emerging from the terminal portion of the parent shoot (P), while the longest lateral shoots (arrows) in crooked aspen crowns arise along the middle of bent parent shoots (P), displaying mesotonic development.

measurement areas (A, B, C, and D), parent shoots were tagged and given a shoot number, a mark was made on the parent shoot at the first selected bud, and all subsequent selected buds were then given a number in sequence based on their nodal position relative to the marked point.

Because of the great variability in crooked shoot morphology, a large sample size (n = 130) was selected. Following the commencement of shoot growth, shorter shoots were eliminated from the study as they became identifiable.

### 3.2.2 Three-Dimensional Digitizing

Starting just after the bud burst (bud swelling and emergence of leaves) and shoot expansion had begun, shoots were digitized using a Polhemus Fastrak® electromagnetic tracking system (Colchester, Vt., USA) (Alméras et al. 2004). The Fastrak® unit consists of a transmitter that emits a magnetic field from a fixed point, a receiver stylus that detects its exact position based on those magnetic fields, and a system electronics unit that contains hardware and software necessary to generate and sense the magnetic fields, compute position and orientation, and interface with the host computer via a USB connection (Polhemus 2002) (Figure 2).

The Fastrak® unit, portable power supply, and laptop computer were taken to the field at approximately weekly intervals through the entire growing season (May 29 to August 22, 2002). Favourable environmental conditions were required to complete measurements, because digitizing was not possible in windy or rainy conditions. Therefore, it was not possible to obtain measurements

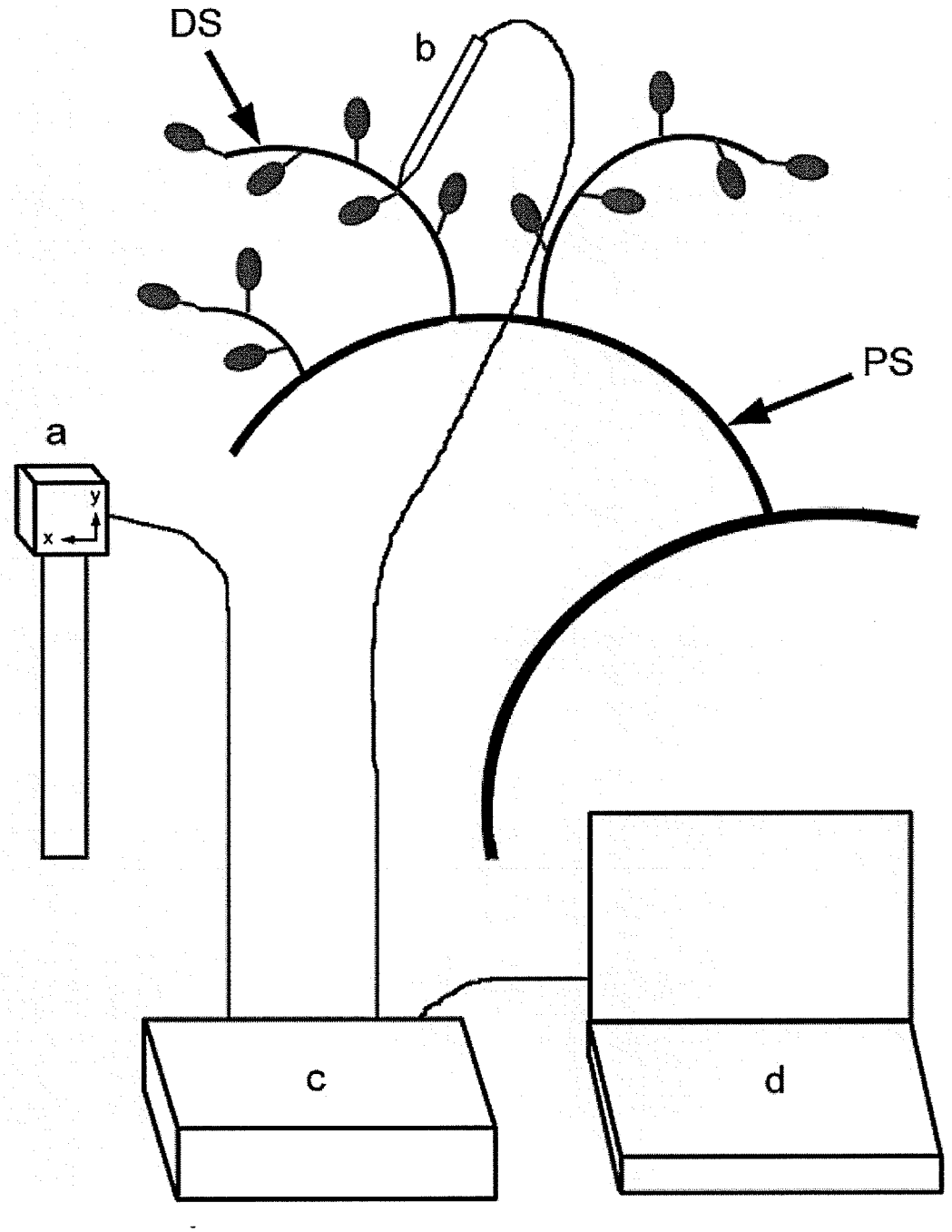



**Figure 2.** Diagram of the Polhemus Fastrak® unit used to digitize nodal locations of crooked aspen daughter shoots (DS) arising from parent shoots (PS). The transmitter (a) emits a magnetic field from a fixed point. The receiver stylus (b) records its exact position in the magnetic field, and relays that information to the system electronics unit (c) which interfaces with a portable laptop computer (d). Diagram is not to scale.

at exactly weekly intervals. On each measurement date, the transmitter was installed on a fixed post located in each of the four measurement areas (A, B, C, and D), and the receiver stylus was used to measure the exact position of each node, beginning at the base of each developing shoot (measured as Cartesian coordinates; X, Y, and Z). These coordinates were recognized and organized using the program DigFoot 2.0 (with permission from Lars Mündermann, University of Calgary, Canada), installed on a Dell Latitude portable laptop computer.

### 3.2.3 Data Analysis

One file was created for each shoot on each measurement date. These files were produced with *.raw formats in DigFoot. This format is not compatible with the modeling software, L-studio (see section 3.2.4). As such, all files were converted to *.txt formats, and were copied to the appropriate L-studio folders for subsequent L-system reconstructions.

### 3.2.4 L-systems and Plant Modeling

Lindenmayer systems, or L-systems (Lindenmayer 1968a; Lindenmayer 1968b; Prusinkiewicz & Lindenmayer 1990), were created with measured data to capture shoot structure and animate its growth and development. The near weekly measurements, similar to the methodology of Prusinkiewicz et al. (1994), facilitated computer animation and visualization.

The plant modeling software, L-studio (University of Calgary, Canada), was used to perform the simulations and visualize the results. L-studio provides a platform for organizing data and transferring it to L-system based simulation

programs (Karwowski & Prusinkiewicz 2004). The initial programming language was written with help from Lars Mündermann (Department of Computer Science, University of Calgary, Canada). Subsequent amendments to the existing programming were made by Brendan Lane (Department of Computer Science, University of Calgary, Canada), and these included standardizing the origin, smoothing the curves, and rendering the simulation in a visually pleasing format. As part of a collaboration with the Department of Computer Science at the University of Calgary data from all digitized shoots was incorporated into a principle component analysis (Brendan Lane, unpublished data). These analyses were used to interpret the morphological variability among shoots of the crooked aspen.

### 3.3 Shoot Ontogeny: Morphological Studies

Studies were conducted to assess differences in shoot morphology between wild-type and crooked *Populus tremuloides* leader shoots as they developed.

#### 3.3.1 Measurements of Shoot Morphology

##### 3.3.1.1 Wild-type and Crooked *Populus tremuloides* Leader Shoots

Prior to bud-break, 40 wild-type aspen terminal buds and 55 crooked aspen buds considered to have a high probability of forming leader shoots were selected. Subsequently, all buds that did not develop into leader shoots, or produced shoots that became injured, were eliminated from the study (see section 3.1.2 for details of procedure).

Measurements for all shoots included parameters related to diameter, length, branching angle from the parent, and numbers of shoot units (number of nodes and associated leaves). The specific parameters are described in Table 1 and illustrated in Figure 3. Measurements were made at approximately biweekly intervals from bud-break to the end of the growing season (June 1, 2004 to August 17, 2004).

In addition to general morphological measurements, a sub-group of 15 wild-type *Populus tremuloides* and 15 crooked *P. tremuloides* leader shoots were also measured to assess taper as they developed. At each measurement date, in addition to basal diameter, the diameter at the midpoint of every second internode that had developed was also measured.

### 3.3.2 Experiments: Manipulating Crooked Aspen Shoot Development

#### 3.3.2.1 Terminal Leaf Removal

The possibility of a terminally produced signal, perhaps hormonal, in influencing shoot bending was evaluated. Ten vigorous crooked aspen leader shoots were selected on June 27, 2004, prior to shoot bending. As a potential source of signal, newly developed, terminal leaves were removed from the shoots with forceps at approximately weekly intervals, as they emerged from the apical region (Leakey & Longman 1986). Every effort was made to minimize damage to the shoot and the shoot apical meristem.

General morphological measurements, as outlined in Table 1, were made on June 27, July 19, August 3, and August 17, 2004. These measurements were

**Table 1.** A description of general morphological shoot measurements made on developing wild-type and crooked *Populus tremuloides* leader shoots biweekly over one growing season.

| Measurement | Description |
|---|---|
| Basal diameter | Shoot basal diameter measurements were made approximately 1 cm above the base of the shoot to avoid the basal shoot swell. Measurements were made to the nearest 1/100 mm using digital vernier callipers. |
| Elevation angle | Angle at which the basal portion of the shoot emerges from the parent shoot with respect to gravity (Figure 3). Angles were measured using a carpenter's tool for calculating roof pitch. |
| Number of leaves | Total number of leaves along the shoot. |
| Number of nodes | Total number of developed nodes along the shoot, with or without associated leaves. |
| Secant angle of termination | Angle from the point of attachment on the parent shoot (the base of the shoot) to the terminal node (shoot tip) with respect to gravity (Figure 3). |
| Shoot length | Shoot length was measured from the base of the shoot to the shoot tip. Length was measured using a small ruler early in development, and a measuring tape later in development. |
| Shoot secant length | Length of the secant between the base of the shoot and the shoot tip. |

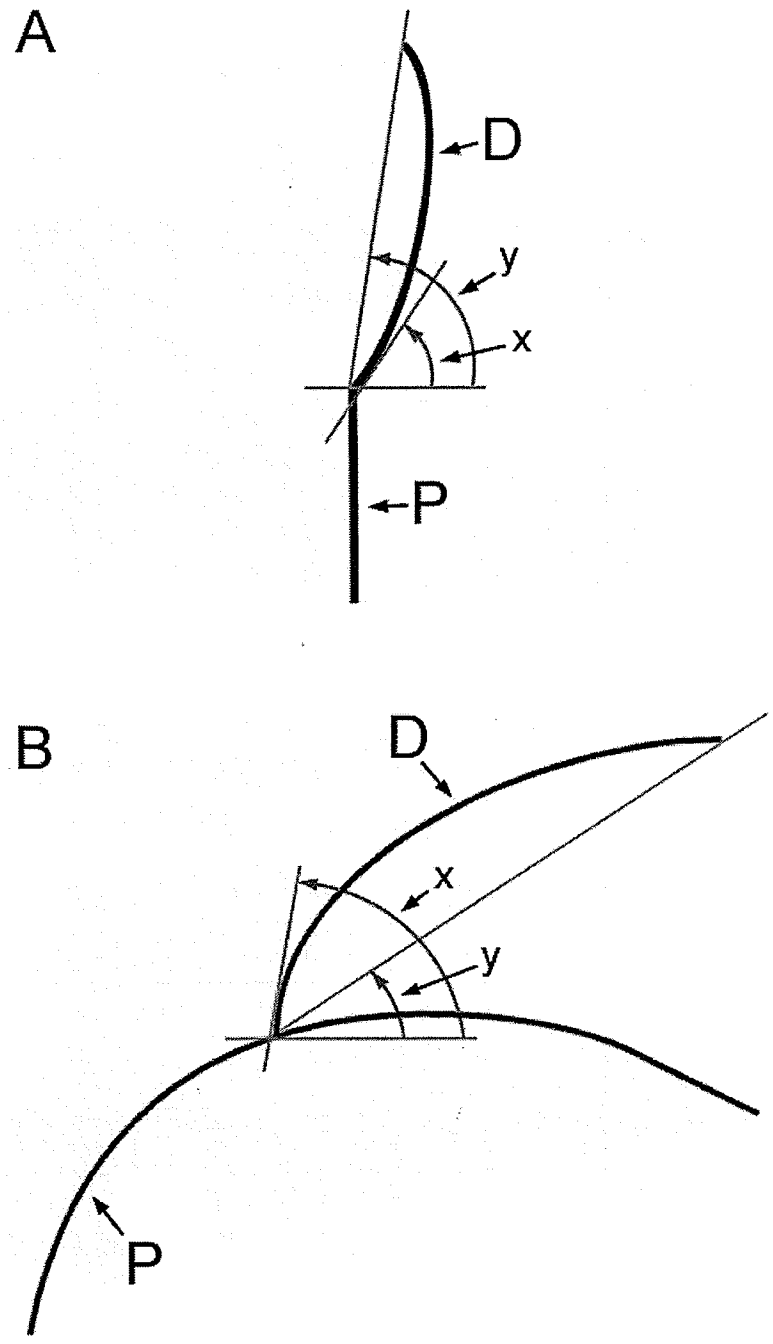



**Figure 3.** Diagrams indicating the elevation angle (x) and the secant angle of termination (y) of daughter leader shoots (D) developing off of parent shoots (P) (see Table 1 for an explanation of the variables). (A) Side-view profile of a wild-type *Populus tremuloides* terminal leader shoot with elevation angle (x) and secant angle of termination (y). (B) Side-view profile of a crooked *P. tremuloides* leader shoot showing the elevation angle (x) and secant angle of termination (y). Angles are measured from horizontal ($0^{o}$).

compared to the morphological measurements of non-experimental crooked leader shoots.

#### 3.3.2.2 Pattern of Daughter Shoot Development along Vertically Reoriented Stems

In the spring of 2003, prior to bud burst 10 crooked aspen pendulous leader shoots from the 2002 growing season were fixed in an upright position. This was accomplished by installing vertically oriented 10 foot lengths of ½ inch electrical conduit in the ground directly adjacent to the experimental shoots (Figure 4). The shoots were fastened to the electrical conduit using a Max Tapener® hand tying machine. The tying material was held in place with duct tape fastened to the conduit.

The pattern of daughter shoot development along the vertically oriented parent shoots was compared with wild-type aspen and regular crooked aspen leader shoots to assess the gravimorphic effect of parent shoot orientation. General morphological measurements (see Table 1) were recorded for the leader shoot (terminal shoot) on all of the vertically reoriented parent shoots over the entire growing season.

### 3.3.3 Shoot Strength and Elasticity

#### 3.3.3.1 In-field Deflection Analysis

In a study designed to test shoot strength and elasticity at the time of crooked aspen shoot bending, 10 crooked and 10 wild-type leader shoots, growing at an angle off vertical (mean secant angle of termination ± SE: wild-type 44.1° ± 3.2°, crooked 44.8° ± 4.4°), were selected. General morphological

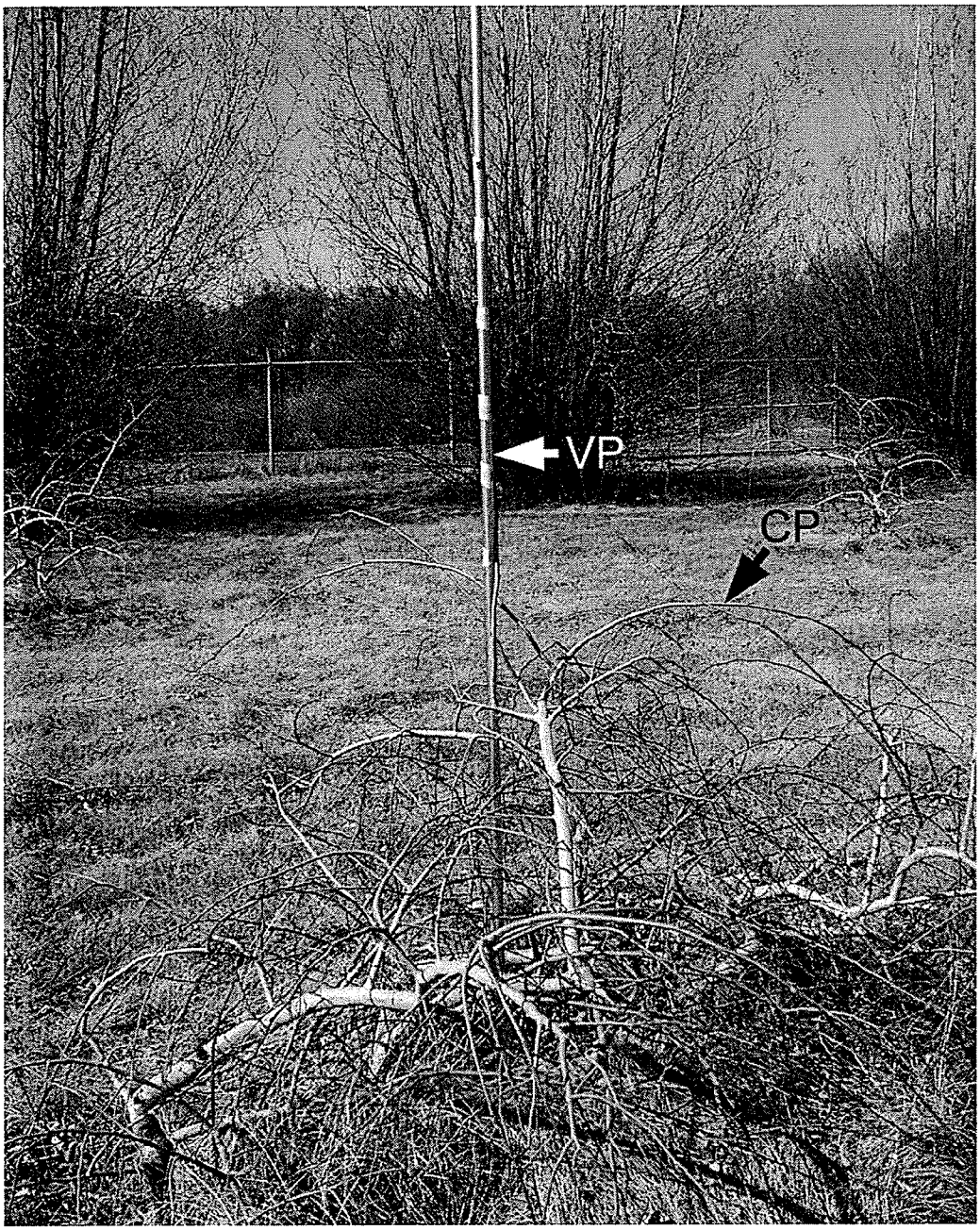



**Figure 4.** A vertically reoriented crooked *Populus tremuloides* parent shoot (VP) alongside a normal crooked parent shoot (CP), as seen April 29, 2003. The orientation of CP was similar to that of VP prior to experimental reorientation. The buds along the parent shoots had not yet burst.

characteristics were measured, and digital photos were taken. Next, a small fishing weight suspended from a 5 cm long wire (total weight of weight and wire = 3.78 g), was attached to each shoot at a point ¾ of the total shoot length from the base. Subsequent shoot deflection was measured immediately (as changes in secant angle) and digital photos were taken from exactly the same position as before the weight was applied using a fixed tripod. Observed shoot deflection was compared between wild-type and crooked aspen shoots in an attempt to identify whether one shoot type was more resistant to deflection (was stronger) than the other shoot type.

#### 3.3.3.2 Laboratory Deflection Analysis

To further evaluate shoot strength and elasticity at the time of crooked aspen shoot bending, 15 wild-type and 15 crooked leader shoots, growing at an angle off vertical (wild-type $43.9^{o} \pm 6.5^{o}$, crooked $47.6^{o} \pm 5.1^{o}$), were harvested June 29, 2005 and brought to the lab for analysis. Shoots were oriented with the upper side of the shoot (as recorded in the field) facing up, and the basal portion of the shoot was held horizontally (at $0^{o}$). Following similar methods to Cannell et al. (1988), the secant angle of termination and the displacement of the free end (terminal end) from the horizontal were measured before and after applying a weight (11.5 g) at a point ¾ of the total shoot length from the base. Resulting shoot deflection was compared between wild-type and crooked *Populus tremuloides* shoots.

### 3.4 Shoot Ontogeny: Anatomical Studies

Along with morphological observations of wild-type and crooked aspen shoots, anatomical studies were performed to identify any differences in structural shoot anatomy that might result in the observed morphology.

#### 3.4.1 Wild-type and Crooked Aspen Shoot Selection

Based primarily on the digitizing study in 2002, it was determined that shoot bending in the crooked aspen usually occurs approximately July 1. Therefore, leader shoots were selected at intervals around the estimated time of bending (June 8, June 22, July 6, and July 20, 2004). To investigate wild-type aspen lateral shoot anatomy, one wild-type shoot oriented at $0^{o}$ (horizontal) and one oriented at $45^{o}$ were harvested on each of the four sampling dates for comparison to wild-type and crooked aspen leader shoots.

Based on a preliminary study in 2003, it was established that replicates were relatively uniform in anatomy, and therefore the sample sizes for anatomical investigations were reduced. On each sample date, four wild-type and four crooked *Populus tremuloides* leader shoots, representative of those observed in the morphological field experiments (section 3.3.1.1), were destructively sampled for anatomical analysis (see section 3.4.3). Subsequently, wild-type and crooked aspen shoot anatomy was compared.

#### 3.4.2 Vertically Trained Crooked Aspen Shoots

To assess whether potential anatomical differences may be a result of bending, an experiment was designed, as a control, to evaluate crooked aspen shoot anatomy when the shoots were grown in an upright orientation, similar to

wild-type aspen leader shoots. Developing crooked aspen shoots were prevented from bending and trained to grow vertically in a structure consisting of a 3 inch diameter cylinder, made of ½ inch hardware mesh oriented and suspended from a vertical scaffold of ¾ inch PVC pipe, anchored by ½ inch electrical conduit (Figure 5). The hardware mesh allowed for minimal obstruction to light and winds, mimicking natural conditions.

The structures were constructed and installed prior to shoot bending. Four shoots were destructively sampled for anatomical analysis on each of three dates (June 22, July 6, and July 21, 2004), which coincided with the last three destructive sampling dates of wild-type and regular crooked aspen shoots (section 3.4.1). Shoot anatomy of vertically oriented crooked aspen shoots was compared to that of both wild-type and non-manipulated crooked aspen. Shoot morphological characteristics were also analyzed to evaluate whether experimental shoots deviated from regular crooked aspen leader shoots.

### 3.4.3 Destructive Shoot Tissue Sampling

All selected shoots were tagged and the upper side of any non-vertical shoot (i.e. leaning or bent shoots) was marked with a permanent marker. The major point of bend of crooked aspen shoots was also marked.

Destructive sampling involved wrapping shoots in wet paper towel and transporting them in a chilled cooler to the lab. In the lab the tissue sampling point was established as the mid-point of relatively vertical shoots (wild-type and young crooked aspen shoots) and the point of bend in bent crooked aspen shoots. The point of bend was usually close to the middle of the shoot.

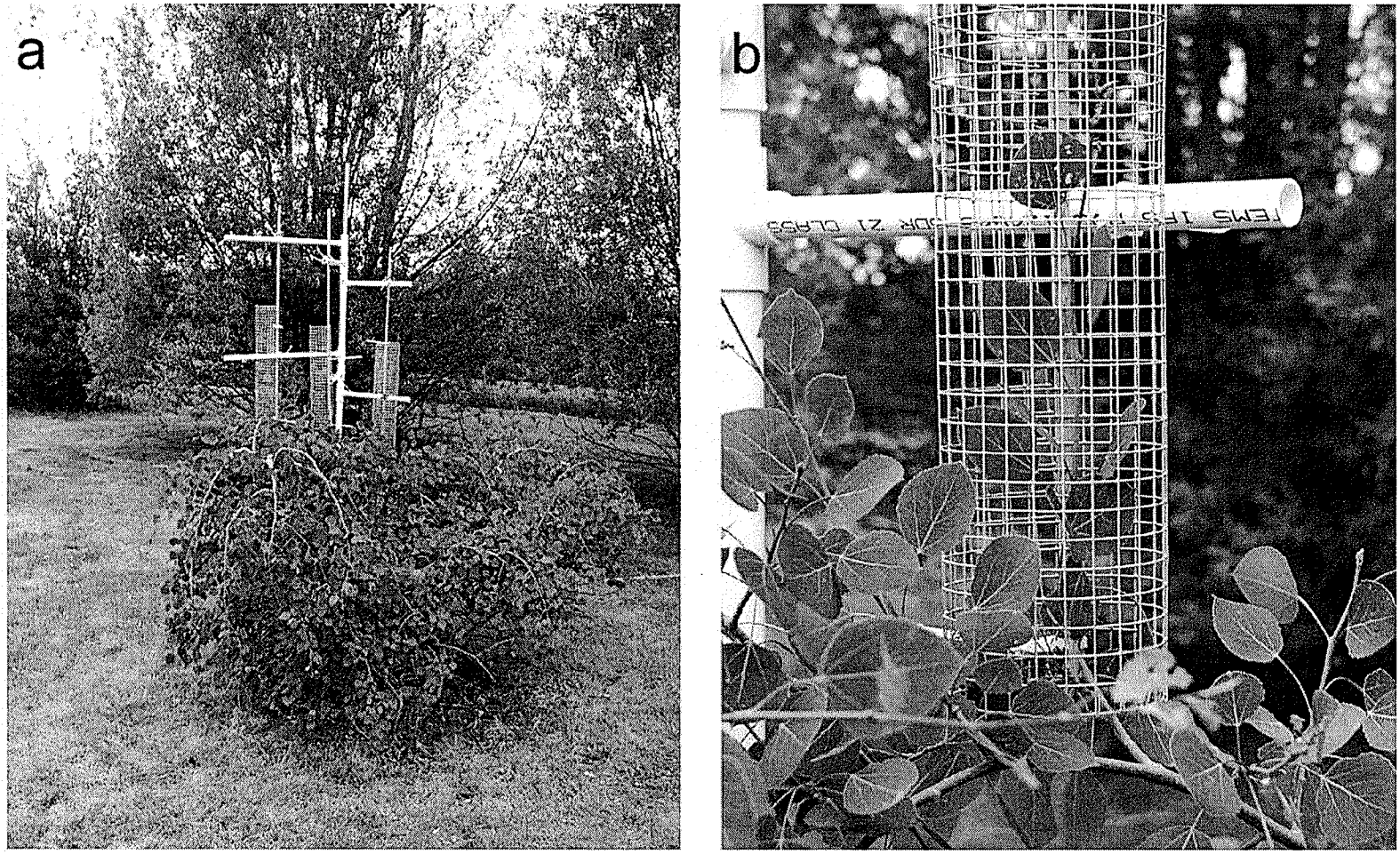



**Figure 5.** (a) Crooked aspen crown with constructed devices for training shoots vertically. (b) Closer view showing a crooked aspen shoot growing vertically through a cylinder of construction mesh (June 22, 2004).

Prior to fixing, the diameter at the sampling point was measured. A double edged disposable razorblade was used to make a very shallow mark along the upper side of bent shoots for future reference. At each sampling point a 40 mm section of shoot was removed using the razorblade and further sliced into 1 to 2 cross sections. These small sections were quickly transferred to fixative (see section 3.4.4.1).

### 3.4.4 Microtechnique

The nature of the woody tissue that was examined proved to be a challenging medium to apply different tissue processing techniques (Yeung 1999; Chaffey 2002b). Microtechnique procedures were refined following a preliminary study in 2003.

#### 3.4.4.1 Fixation

Tissue sections from each shoot were placed in labelled 10 ml vials and fixed in a modified Karnovsky's fixative consisting of 2.5% glutaraldehyde and 1.6% paraformaldehyde buffered with 0.05 M potassium phosphate at pH 6.9 (Yeung 1999; Stasolla et al. 2002). To remove air spaces for even infiltration, vials were vacuum infiltrated 4 times at 25 Hg in a vacuum sealed chamber. Sample vials were then placed on a rotator at room temperature for 24 hours followed by 24 hours in a refrigerator (4°C). Subsequently, tissue was washed with two changes of cold 0.05 M potassium phosphate buffer, 20 minutes each, to remove all fixative.

### 3.4.4.2 Dehydration

Tissue dehydration was accomplished by replacing aqueous buffer with pure methyl cellosolve (2-methoxyethanol) and incubating for 24 hours at 4°C. This step was repeated, and was followed by two changes of absolute ethanol, 24 hours each (modified from Feder & O'Brien 1968).

### 3.4.4.3 Infiltration

Following dehydration, shoot tissue was infiltrated with Historesin (Leica Microsystems Nussloch GmbH, Nussloch, Germany), which is a glycol methacrylate (GMA) infiltration and embedding medium (Feder & O'Brien 1968). The infiltration solution was prepared by dissolving 5 g activator (dibenzolperoxide) in 500 ml basic resin ((2-Hydroxyethyl)-methacrylate) (1%) at room temperature. To ensure even penetration, infiltration was carried out as a graded series of changes of absolute ethanol:infiltration solution: 75:25, 50:50, 25:75, followed by two changes in 100% infiltration solution, incubated and rotated at 4°C for 48 hours after each change (Yeung 1999; modified from Ruzin 1999). Vials containing shoot tissue and the final change of infiltration solution were stored at 4°C, prior to embedding.

### 3.4.4.4 Embedding

Following infiltration, individual shoot sections (1 to 2 mm thick) were carefully removed from vials using a pair of fine forceps. Each section was oriented with the surface to be cut facing down, in individual wells of a polyethylene mould tray. Embedding solution was prepared by mixing 15 ml of infiltration solution with 1 ml hardener (component 3, of the Leica Historesin

embedding kit), and 0.2 ml polyethylene glycol (PEG). The hardener begins the polymerization immediately, and the PEG increases the "stickiness" of the section (Yeung 1999). The embedding solution was transferred immediately to the mould tray with a Pasteur pipette, filling the lower portion of the well. A plastic block holder was placed in the tray, over the well, and the mould was topped up with embedding solution applied through the central hole in the plastic block holder. This final step excluded air from the surface of the mould which interferes with the polymerization process (Yeung 1999; Ruzin 1999). All steps were repeated until all of the wells were processed. Embedded wells were left to polymerize for 48 hours at room temperature before sectioning.

**3.4.4.5 Sectioning**

After polymerization, glycol methacrylate forms a transparent matrix of high tensile strength (O'Brien & McCully 1981) that is readily sectioned on a retracting rotary microtome (Yeung 1999; Ruzin 1999). For this study, sectioning was carried out with Ralph type glass knives on a Leica RM2145 rotary microtome with a retractable return stroke.

Knives were installed at a 7° incline. A small amount of liquid PEG was painted on the edges of the block to improve ribbon formation (Yeung 1999). Thin sections (3-5 µm), either individually or as a ribbon, were placed in deionized distilled water on a glass slide, and dried on a slide warmer at 40°C. Although sections generally adhere well to clean glass slides (O'Brien & McCully 1981; Yeung 1999), it was observed that some of the thick, dense sections of

older woody tissue did not completely adhere to the glass (Feder & O'Brien 1968).

#### 3.4.4.6 Staining

Staining of glycol methacrylate embedded sections is much simpler than paraffin sections, as the embedding medium does not interfere with the staining procedure and therefore it is not necessary to remove it (Yeung 1999). The sections were stained with a variety of aqueous stains (O'Brien & McCully 1981; Ruzin 1999; Chaffey 2002b).

##### 3.4.4.6.1 Periodic Acid-Schiff – Toluidine Blue O Schedule

For general histological examinations, the sections were stained with the periodic acid-Schiff (PAS) – toluidine blue O (TBO) schedule (Stasolla et al. 2002). The PAS–TBO schedule is designed so that cell wall components like starch, pectin, and hemicellulose are stained red with the PAS reaction, and TBO (a metachromatic stain) stains most primary walls pink and lignified secondary cell walls are stained blue-green (O'Brien & McCully 1981).

Numerous slides were stained at once using a staining dish and slide rack (Ruzin 1999). Slides were placed in 0.1% periodic acid for 15 minutes and were then washed under cold running water for 5 minutes. Slides were transferred to a staining dish containing Schiff's reagent for 30 minutes, excluding any light from the solution with tinfoil. Next, the slides were washed under running water for 8 minutes (modified from Feder & O'Brien 1968). Slides were then counterstained by immersing the slides in 0.05% (w/v) TBO in a benzoate buffer, pH 4.4 (Feder & O'Brien 1968; O'Brien & McCully 1981; Stasolla et al. 2002), for

3 minutes. Next, slides were rinsed under running water until the plastic was nearly free of stain (5-10 minutes). Slides were allowed to air dry for 24 hours in a dust free area.

**3.4.4.6.2 Safranin O – Astra Blue Schedule**

Safranin O counterstained with astra blue is a common differential staining technique used to distinguish between lignified and unlignified tissues (Vazquez-Cooz & Meyer 2002; Chaffey 2002b). This procedure has been used to identify the purely cellulosic gelatinous (G) layer characteristic of tension wood in woody angiosperms (Srebotnik & Messner 1994; Alméras et al. 2004), including young *Populus* spp. shoots (Jourez et al. 2001). Safranin O stains lignified secondary cell walls pink while astra blue stains cellulose and non-lignified cell walls, including the G-layer, blue. This staining schedule provides a definite presence or absence answer to the occurrence of TW in shoot sections.

Using a slide rack, multiple slides were transferred to a staining dish containing safranin O solution (Sigma catalog no. HT90432) diluted 1:10, v/v with deionized distilled water for 3 minutes. Slides were then washed under cold running water for 4 minutes until the plastic was free of background stain. Subsequently, slides were counterstained with 0.5% astra blue (0.5 g astra blue (Sigma catalog no. 73580) in 100 ml of aqueous 2% (w/w) tartaric acid) for 3 minutes. Slides were then rinsed (1 minute), heated on a slide warmer for 30 seconds at 40°C, and allowed to air dry overnight (modified from Chaffey 2002b).

For viewing purposes, the sections were mounted on slides in Cytoseal™ 60 mounting medium (Richard-Allan Scientific no. 8310-16), and a coverslip was

applied. The slides were placed on a slide warmer (30°C) overnight to aid in polymerization and to minimize air bubble formation.

### 3.4.5 Histological Analysis

#### 3.4.5.1 Light Microscopy and Photomicroscopy

A Leica DMRE (Leica Microsystems, Wetzlar, Germany) light microscope equipped with a Leica DC500 digital camera was used to examine and photograph prepared slides. The oldest shoot sections were examined first as differences in anatomy are often easiest to identify in more mature tissue (Yeung 1999). Once structures of interest were recognized, younger sections were examined for comparison.

Sections of the different shoot types (see sections 3.4.1 and 3.4.2) were examined for the presence, absence, quantity, and quality of the following structural components: xylem tissue (Mauseth 1988; Okuyama et al. 1994; Mellerowicz et al. 2001; Donaldson 2001; Plomion et al. 2001), phloem fibres (Mauseth 1988; Den Outer 1993; Niklas 1999), and anomalous tissues such as tension wood (Wilson & Archer 1977; Boyd 1977; Fisher & Stevenson 1981; Jourez et al. 2001; Alméras et al. 2004).

The relative amount of each major tissue type (cortex, phloem, xylem, and pith) found in the observed sections was measured using an ocular micrometer, calibrated to a stage micrometer (O'Brien & McCully 1981). Sections were measured from one side to the other, typically the upper to the lower side, where applicable. Measurements of young shoot sections were more difficult as most tissues were immature and the pith was quite erratic, resulting in non-circular

arrangements of each tissue type. The tissue was not eccentric, and therefore only one representative shoot section from each of the shoot types analyzed (sections 3.4.1 and 3.4.2) on the first three sample dates was measured. For the fourth and final sample date all four sampled shoots from each treatment (sections 3.4.1 and 3.4.2) were measured, and a mean was calculated.

### 3.5 Growth Analyses and Young's Modulus

Young's modulus (*E*) (section 2.3.3.2) measures the rigidity of woody plant stems (Cannell & Morgan 1987). Using regression analysis, Cannell and Morgan (1987) found that moisture content (MC) could account for about half of the variation in *E* ($r^2 = 0.49$). In effect Young's modulus decreased with an increase in stem moisture content. Therefore, as an indirect estimate of Young's modulus, moisture content (on a dry weight basis) was measured in 12 wild-type trembling aspen leader shoots and 12 crooked aspen leader shoots. Shoots were harvested on October 1, 2004, after the growing season was completed, and any remaining leaves were removed. Shoot green weight ($W_g$) and oven dried weight ($W_d$ – dried at 80°C until there was no change in weight) were both measured. Moisture content (MC) on a dry basis was calculated as

(1) $$MC = (W_g - W_d)/W_d \times 100\%$$ (Simpson 1993).

Cannell and Morgan's (1987) Young's modulus was then estimated from the regression equation on MC over the range of 60-150% as

(2) $$E = 7.91 - 0.0447(MC)$$

Moisture content and Young's modulus data from wild-type and crooked aspen shoots were subsequently compared statistically (see section 3.6).

### 3.6 Statistical Analyses

To compare quantitative architectural patterns of wild-type aspen to crooked aspen, data were analyzed using standard statistical techniques including correlation analysis, comparisons of means (t-tests), and linear regression (Remphrey & Pearn 2003). Where slopes were similar in the linear regression analysis, analysis of covariance (ANCOVA) was performed to identify potential differences in the intercepts. Data were analyzed using Statistical Analysis Systems 8 (SAS Institute Inc., Cary, NC, 1999-2001).

Studies where the experimental units are observed repeatedly over the course of time are called repeated measures experiments. Repeated measures analysis of variance (ANOVA) was performed using the GLM procedure of SAS. This procedure gives values for a particular measurement over time after having adjusted for the fact that the values have been repeatedly obtained from a group of the same shoots.

# 4.0 RESULTS

## 4.1 Location, Timing, and Form of Crooked Aspen Shoot Bending

### 4.1.1 L-system Shoot Reconstruction Model

L-system modeling of crooked aspen shoot elongation captured the progressive stages of bending over the growing season (Figure 6). L-studio provided visual images of the progressive changes from one measurement date to the next. An examination of a representative crooked aspen leader shoot revealed that early in the season, while the shoot was still quite small, its orientation was relatively vertical (Figure 6a). Several weeks later, as the internodes were extending, the terminal half of the shoot developed slightly off vertical, and by July 4, 2002 the majority of the shoot showed increasing lean (greater angle off vertical), while the lower, fully extended internodes maintained a smaller angle off vertical (Figure 6b). Subsequent and progressive shoot bending occurred over a relatively short period of time in early July. Initially, shoot orientation ranged from nearly vertical in the basal region, to horizontal approximately midway along the shoot, to slightly more vertical near the shoot tip (Figure 6c). The midpoint along the shoots measured on July 9 was the greatest point of bend through the remaining measurement dates. Later, shoot bending became more pronounced with an increasingly long pendulous segment extending downwards from the bending point, ending with the youngest, most recently formed internodes of the shoot tip oriented at some angle up, away from the gravitational stimulus (Figure 6d). With increasing length of the bending

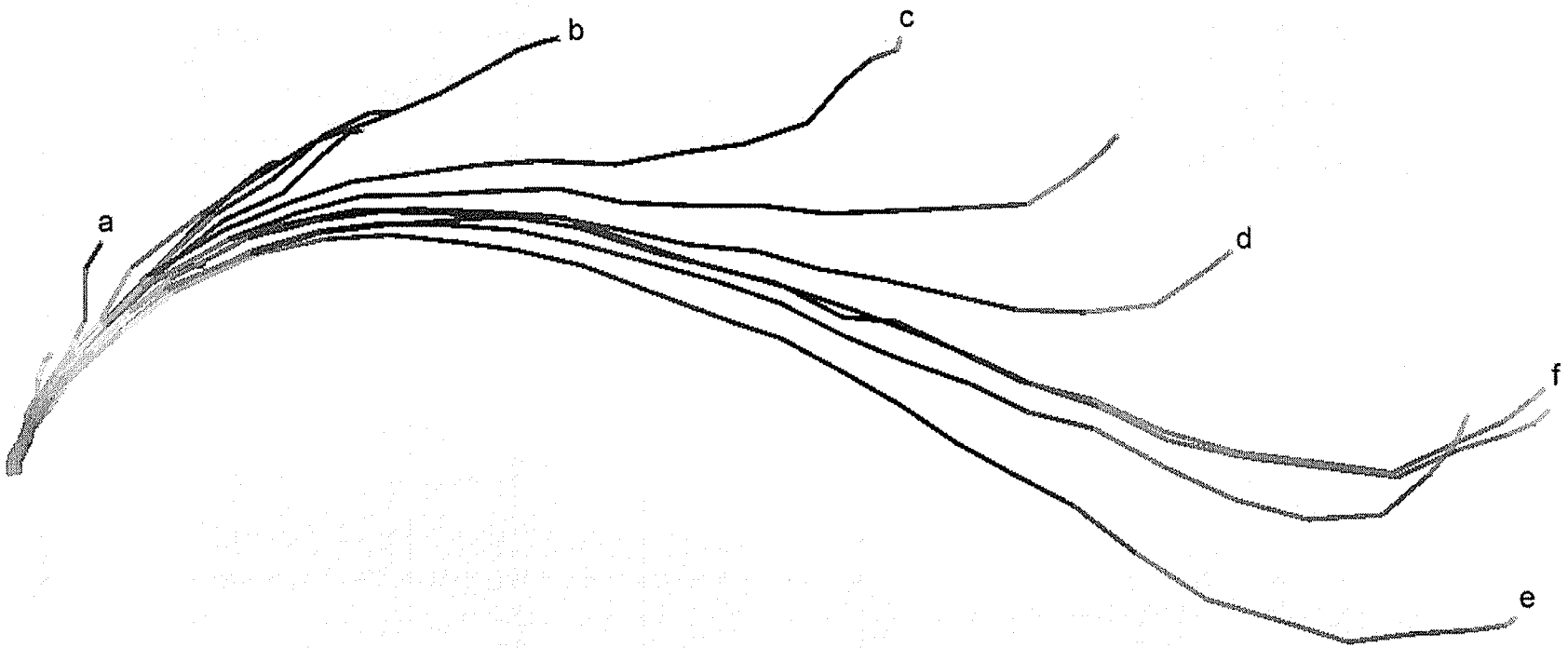



**Figure 6.** L-system reconstruction model of an example of a crooked aspen shoot development over one growing season. All measurement dates are superimposed; each is represented by one continuous line. Coloured increments mark analogous regions on the shoot from date-to-date. Only selected dates are labelled for clarity and to indicate the sequence of development (a = June 12, b = July 4, c = July 9, d = July 17, e = August 6, and f = August 15, 2002). Courtesy of Brendan Lane (Department of Computer Science, University of Calgary, Canada).

shoots, it was observed that the entire shoot lowered slightly, in relation to gravity (Figure 6e). Minute shoot reorientation upward was observed in the final two measurement dates, with the shoot becoming slightly less pendulous (Figure 6f); however, upwards reorientation was not characteristic of all crooked aspen shoots.

A principal component analysis performed on the spatial coordinates of all digitized shoots revealed that there was variation in the pattern of shoot bending (Figure 7). First, the degree of bending, or droop, varied among shoots of similar lengths (Figure 7a). The major point of bend occurred at a variety of positions, from near the base to two thirds the length of the shoot. Next, shoot length affected bending in that long shoots were more pendulous than short shoots. Comparing bent shoots of different lengths, the basal portion of shorter shoots was maintained in a more vertical fashion than that of longer shoots (Figure 7b). On average, the major point of bend was at approximately the same absolute distance along both long and short shoots; however, additional extension growth in the long shoots provided a proportionally longer pendulous portion. Finally, there was a range in the tropic orientation of the shoot tip, some showing strong negative gravitropism, while others had a weaker response (Figure 7c).

## 4.2 Shoot Ontogeny: Comparison of Wild-type and Crooked Aspen

### 4.2.1 Correlations Among Morphological Variables

All morphological variables measured (Table 1) were significantly correlated for wild-type shoots (Table 2a). The same was true for crooked aspen shoots with the exception of the angle of elevation (Table 2b). Not surprisingly,

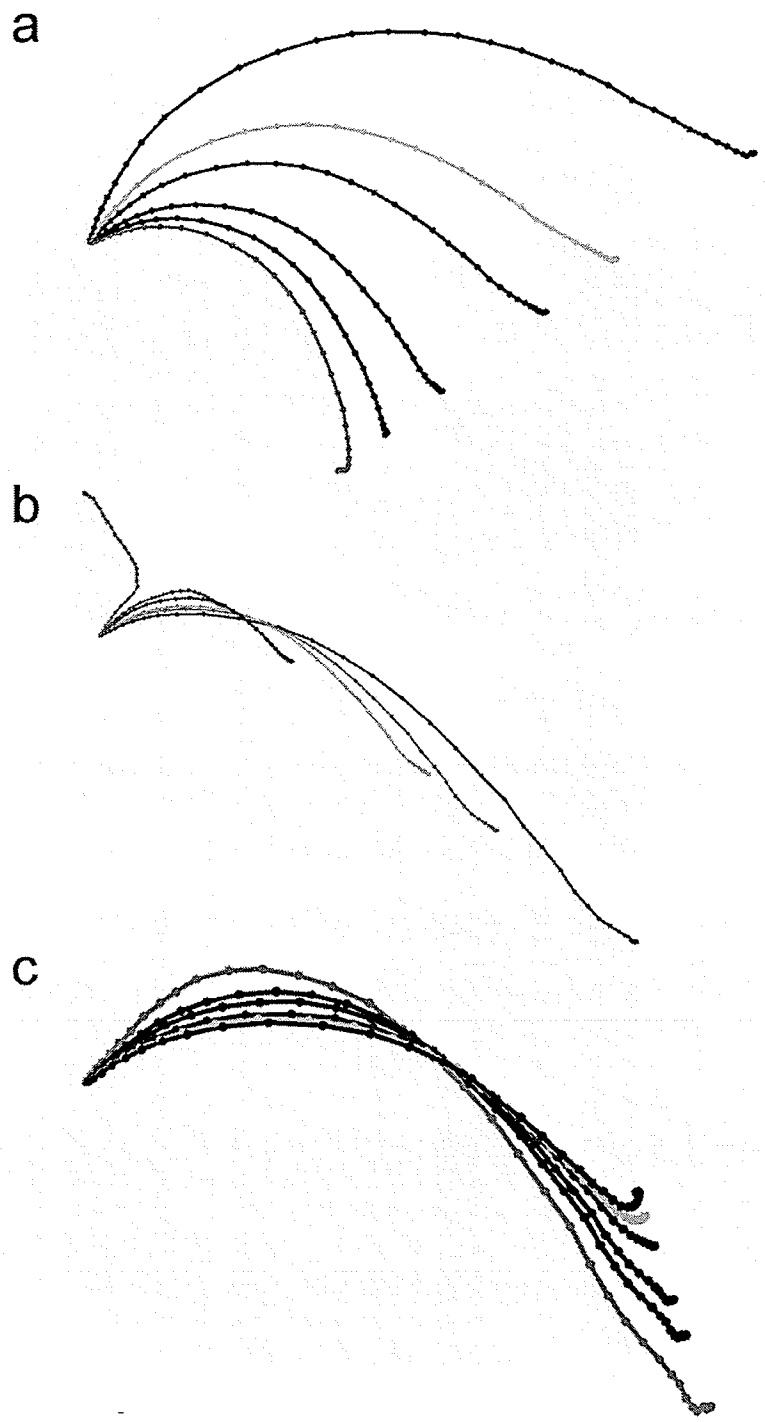



**Figure 7.** Visual representations of the variation in shoot morphology based on a principal component analysis of all shoots digitized on August 1, 2002. L-system reconstructed shoots represent a range from -2 to 2 standard deviations from the mean of each principle component. (a) "droop", (b) shoot length, and (c) tropic response of the shoot tip. Courtesy of Brendan Lane (Department of Computer Science, University of Calgary, Canada).

**Table 2.** Matrix of correlations coefficients for all morphological variables measured on current-year shoots over one growing season.

**a.** Wild-type *Populus tremuloides* (n = 81).

| | 1. | 2. | 3. | 4. | 5. | 6. | 7. |
|---|---|---|---|---|---|---|---|
| 1. Basal diameter | - | | | | | | |
| 2. Shoot length | 0.97* | - | | | | | |
| 3. Secant length | 0.97* | 1.00 | - | | | | |
| 4. Elevation angle | -0.32* | -0.33* | -0.33* | - | | | |
| 5. Secant angle | -0.32* | -0.33* | -0.33* | 1.00 | - | | |
| 6. Number of nodes | 0.97* | 0.98* | 0.98* | -0.32* | -0.32* | - | |
| 7. Number of leaves | 0.96* | 0.96* | 0.96* | -0.31* | -0.31* | 0.99* | - |

* $P < 0.01$

**b.** Crooked *Populus tremuloides* (n = 154).

| | 1. | 2. | 3. | 4. | 5. | 6. | 7. |
|---|---|---|---|---|---|---|---|
| 1. Basal diameter | - | | | | | | |
| 2. Shoot length | 0.95* | - | | | | | |
| 3. Secant length | 0.95* | 0.99* | - | | | | |
| 4. Elevation angle | -0.12 | -0.12 | -0.13 | - | | | |
| 5. Secant angle | -0.41* | -0.48* | -0.43* | 0.71* | - | | |
| 6. Number of nodes | 0.95* | 0.96* | 0.94* | -0.11 | -0.51* | - | |
| 7. Number of leaves | 0.87* | 0.91* | 0.89* | -0.08 | -0.51* | 0.95* | - |

* $P < 0.01$

the strongest correlations, all positive, were among basal diameter, shoot length, shoot secant length, number of nodes, and number of leaves. Overall, with the increased production of nodes and associated leaves, shoot length increased, resulting in an increase in basal diameter. Shoot elevation angle (wild type), and secant angle (wild type and crooked) were negatively correlated with shoot length. These angles decreased (shoot orientation became more oblique) as shoot length increased (Figure 6).

### 4.2.2 Shoot Patterns Over Time

Each measured morphological variable was analyzed separately to determine whether there was a significant difference in a particular morphological trait between wild-type *Populus tremuloides* and crooked *P. tremuloides* shoots (tree-type effect). There was no significant tree-type effect for shoot length ($P = 0.83$) or secant length ($P = 0.66$) (Figures 8a and b), indicating that wild-type and crooked aspen leader shoots had similar lengths. Also, wild-type and crooked aspen shoots were not significantly different in number of nodes ($P = 0.11$) or number of leaves ($P = 0.30$) (Figures 8c and d).

There was a significant difference between wild-type and crooked *P. tremuloides* shoot basal diameter ($P < 0.01$), elevation angle ($P < 0.01$), and secant angle ($P < 0.01$) when observed across all dates (Table 3). A significant difference in these variables indicates that tree-type effect changed significantly from measurement date to measurement date (Figure 9). In particular, the basal diameter of crooked aspen shoots was greater than wild-type aspen shoots (Table 3). Wild-type aspen shoots were oriented significantly more vertical, with

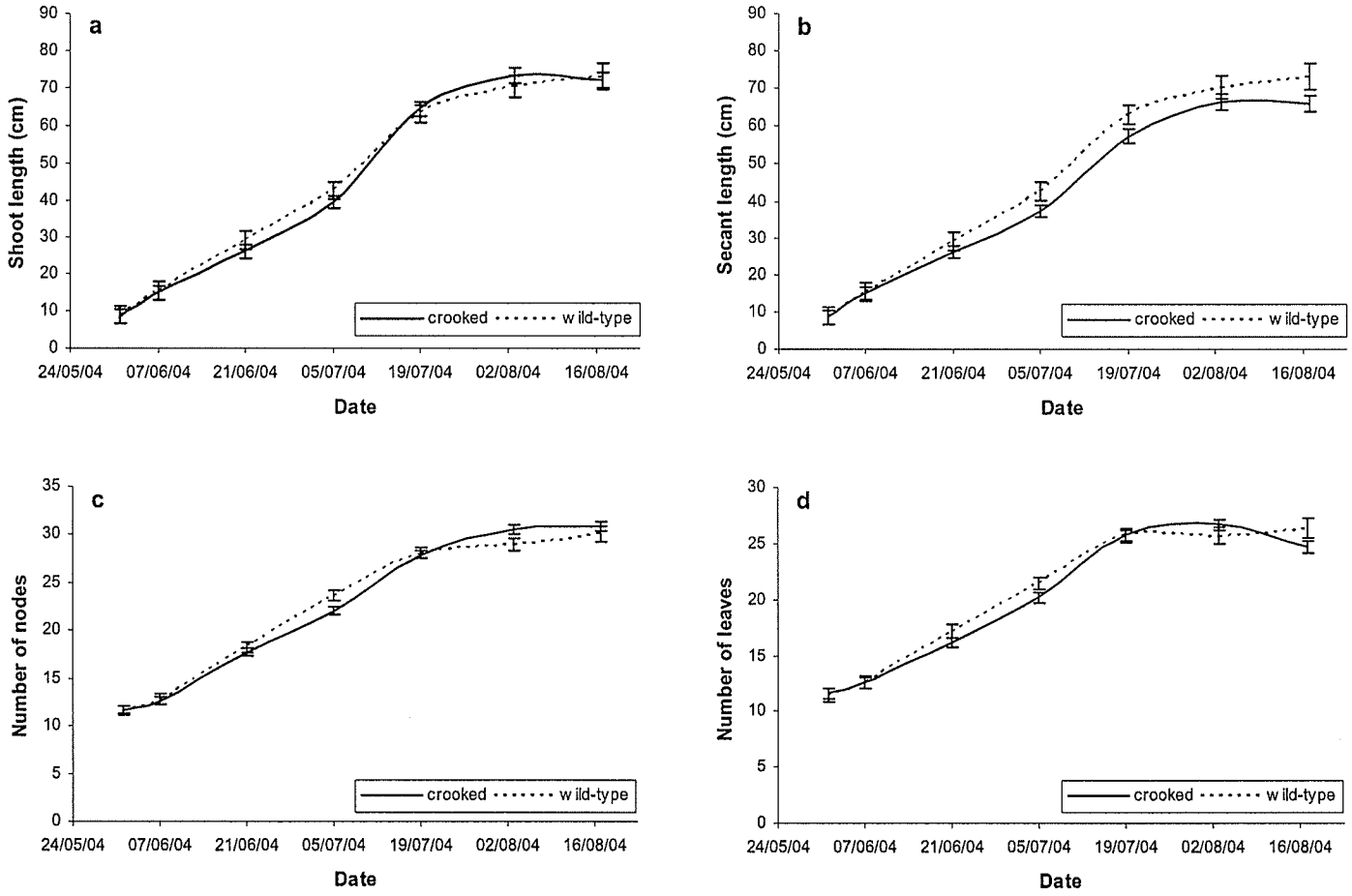



**Figure 8.** A comparison of morphological variables for wild-type and crooked *Populus tremuloides* shoots measured over the growing season. Values indicated are least squares means (LS mean) and standard errors (SE) from a repeated measures analysis of variance. a, shoot length; b, secant length; c, number of nodes; d, number of leaves.

**Table 3.** Repeated measures analysis of tree-type effect (wild-type *Populus tremuloides* vs. crooked *P. tremuloides*) on morphological variables measured over the growing season of 2004.

| | | | Tree type effect probability (P) level [b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | All | Sorted by date [c] | | | | | | |
| Variable | Wild-type [a] | Crooked [a] | dates | June 1 | June 7 | June 21 | July 5 | July 19 | August 3 | August 17 |
| Basal diameter (mm) | 5.02 ± 0.31 | 5.91 ± 0.21 | <0.01 | 0.42 | 0.21 | 0.02 | 0.02 | <0.01 | <0.01 | <0.01 |
| Shoot length (cm) | 43.22 ± 2.73 | 42.69 ± 1.94 | 0.83 | 0.90 | 0.88 | 0.31 | 0.28 | 0.66 | 0.42 | 0.81 |
| Secant length (cm) | 43.22 ± 2.71 | 39.45 ± 1.93 | 0.66 | 0.90 | 0.88 | 0.32 | 0.07 | 0.06 | 0.29 | 0.07 |
| Elevation angle (°) | 75.18 ± 5.27 | 55.66 ± 3.76 | <0.01 | 0.37 | 0.02 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Secant angle (°) | 75.18 ± 5.87 | 31.16 ± 4.19 | <0.01 | 0.56 | 0.13 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Number of nodes | 21.91 ± 0.74 | 21.89 ± 0.53 | 0.11 | 0.99 | 0.83 | 0.44 | 0.02 | 0.78 | 0.05 | 0.41 |
| Number of leaves | 20.08 ± 0.74 | 19.68 ± 0.52 | 0.30 | 0.83 | 0.97 | 0.18 | 0.08 | 0.98 | 0.27 | 0.10 |

[a] Values are least squares mean (LS mean) ± standard error (SE) for the entire tree type effect across all dates.

[b] Significance levels (P) based on tests of hypothesis for repeated measures analysis of variance (ANOVA).

[c] Significance levels (P) of the tree type x date effect sorted by measurement date for each variable.

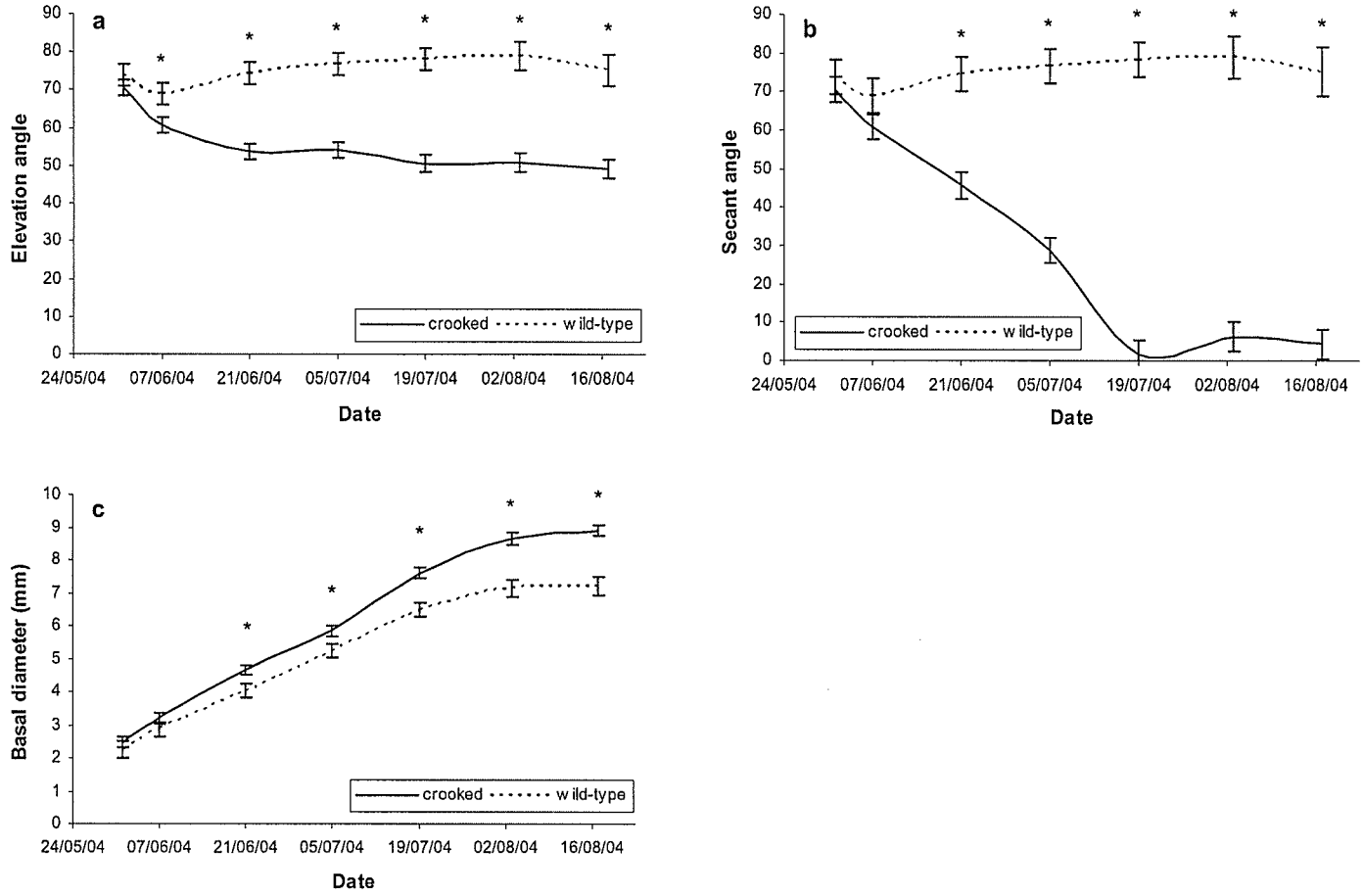



**Figure 9.** A comparison of morphological variables for wild-type and crooked *Populus tremuloides* shoots measured over the growing season. Values indicated are least squares mean (LS mean) and standard error (SE) from a repeated measures analysis of variance. a, angle of elevation; b, secant angle of termination; c, basal diameter. * indicates significant difference ($P < 0.05$) in that variable between wild-type and crooked *P. tremuloides* shoots.

greater elevation and secant angles, compared to those of crooked aspen shoots (Table 3). Bending was apparent in crooked aspen shoots as revealed by more vertical basal elevation angles compared to the less vertical termination angles (secant angles). Therefore, in wild-type aspen shoots, elevation and secant angles were similar and shoots were relatively straight and non-bent (Table 3).

Variables that resulted in significant tree-type effects were further analyzed to determine on which dates the differences occurred. For basal diameter there was no significant difference between wild-type and crooked aspen shoots on the first two measurement dates (June 1 and 7, 2004) (Table 3). However, on June 21, just prior to shoot bending, and all subsequent measurement dates, there was a significant difference between each shoot type, with crooked aspen shoots having the greatest diameter. Moreover, basal diameter became increasingly different between wild-type and crooked aspen shoots over time (Figure 9c). An examination of individual dates for shoot elevation angle (Table 3), revealed that wild-type shoots had significantly larger elevation angles than crooked aspen shoots on the second measurement date, June 7, 2004. The significant difference was maintained with relatively little change through the remainder of the season (Figure 9a). Shoot secant angle of termination was significantly different between wild-type aspen and crooked aspen shoots on June 21, 2004 (Table 3), at which time shoot leaning marked the early stages of shoot bending. During and after shoot bending, wild-type and crooked *P. tremuloides* shoot secant angles were very different for all dates (Table 3). Crooked aspen shoot secant angle reached a minimum before the

end of the season (approximately July 19, 2004) (Figure 9b), marking the approximate end of shoot bending in 2004.

### 4.2.3 Shoot Length by Basal Diameter Relationships Over Time

As was previously indicated, shoot length and basal diameter were significantly correlated for both wild-type and crooked *Populus tremuloides* (section 4.2.1), each variable increasing over time (section 4.2.2). The rate of increase in basal diameter with respect to shoot length over time was examined. A comparison of linear regressions of the least squares mean (LS mean) values for each variable from each measurement date revealed a significant difference in the slopes of wild-type and crooked aspen shoots ($P < 0.01$) (Figure 10). Crooked aspen shoots had a greater rate of increase in basal diameter per unit shoot length compared with wild-type aspen shoots.

### 4.2.4 Analysis of Shoot Taper Over Time

For each measurement date a repeated measures ANOVA was used to provide an overall probability value for the tree-type effect on diameter across all measured internodes (Table 4). In general, except for the first date, there was a significant difference in the interaction of diameter vs. internode number between tree types. In other words, shoot taper was different between each tree type, crooked aspen developing with increasingly more tapered shoots compared to the wild type.

Shoot taper was illustrated by sorting the model by internode number and reporting the means (± SE) (Figure 11) and P-values (Table 4) for each measured internode. Regardless of the date, shoots of both types were always

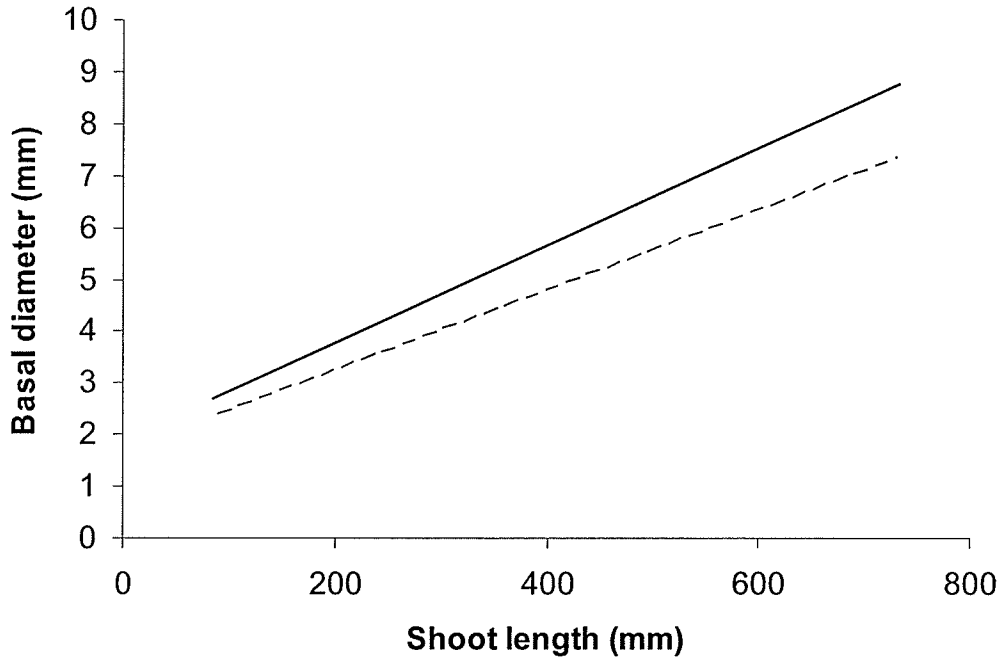



**Figure 10.** Relationship between basal diameter (y) and shoot length (x) of wild-type *Populus tremuloides* (broken line: $y = 1.70 + 0.0077x$; $R^2 = 0.97$, n = 91) and crooked *P. tremuloides* (solid line: $y = 1.90 + 0.0089x$; $R^2 = 0.95$, n = 168) measured at nearly biweekly intervals over one growing season. Slopes were significantly different ($P < 0.01$).

**Table 4.** Repeated measures analysis of tree-type effect (wild-type *Populus tremuloides* vs. crooked *P. tremuloides*) on shoot diameter measured at every second internode along developing shoots over the growing season of 2004.

| | June 1 | June 7 | June 21 | July 5 | July 19 | August 3 | August 17 |
|---|---|---|---|---|---|---|---|
| Overall shoot diameter (mm) [a] | | | | | | | |
| Wild-type shoots | 1.36 ± 0.16 | 1.64 ± 0.21 | 2.33 ± 0.23 | 2.88 ± 0.31 | 3.42 ± 0.54 | 3.52 ± 0.81 | 3.73 ± 0.86 |
| Crooked shoots | 1.48 ± 0.17 | 1.78 ± 0.20 | 2.54 ± 0.23 | 3.16 ± 0.32 | 3.87 ± 0.62 | 4.31 ± 0.88 | 4.63 ± 0.90 |
| Tree-type effect across all internodes [b] | 0.11 | 0.02 | < 0.01 | 0.07 | < 0.01 | < 0.01 | < 0.01 |
| Tree type effect sorted by internode [c] | | | | | | | |
| Internode 1 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| Internode 3 | 0.25 | 0.43 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| Internode 5 | 0.52 | 0.61 | < 0.01 | 0.02 | < 0.01 | < 0.01 | < 0.01 |
| Internode 7 | 0.89 | 0.59 | 0.33 | 0.12 | < 0.01 | < 0.01 | < 0.01 |
| Internode 9 | 0.62 | 0.35 | 0.66 | 0.25 | 0.02 | < 0.01 | < 0.01 |
| Internode 11 | 0.10 | 0.17 | 0.93 | 0.90 | 0.02 | < 0.01 | < 0.01 |
| Internode 13 | | 0.02 | 0.31 | 0.91 | 0.83 | < 0.01 | < 0.01 |
| Internode 15 | | | 0.75 | 0.02 | 0.01 | < 0.01 | < 0.01 |
| Internode 17 | | | < 0.01 | 0.16 | 0.01 | < 0.01 | < 0.01 |
| Internode 19 | | | | 0.85 | < 0.01 | < 0.01 | < 0.01 |
| Internode 21 | | | | 0.29 | 0.02 | < 0.01 | < 0.01 |
| Internode 23 | | | | | 0.18 | < 0.01 | < 0.01 |
| Internode 25 | | | | | 0.36 | 0.04 | 0.55 |
| Internode 27 | | | | | 0.89 | 0.17 | 0.69 |
| Internode 29 | | | | | 0.27 | 0.87 | 0.29 |
| Internode 31 | | | | | | 0.74 | < 0.01 |

[a] Least squares means (LS means) ± standard errors (SE) for shoot diameter calculated from a repeated measures analysis of variance (ANOVA).

[b] Significance levels (P) for the interaction over all internodes, based on repeated measures ANOVA.

[c] Significance levels (P) for individual measured internodes, based on repeated measures ANOVA sorted by internode.

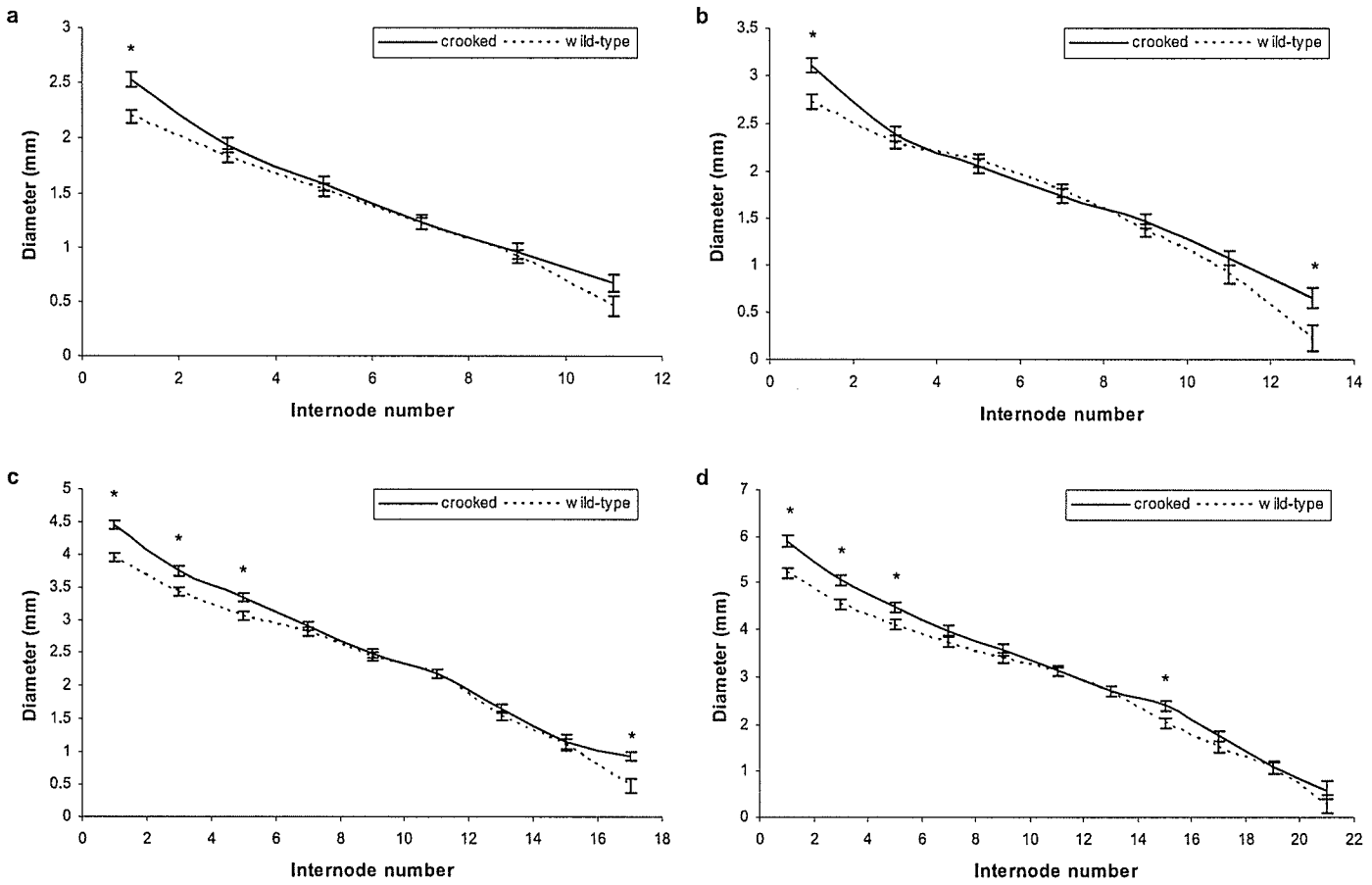



**Figure 11.** Shoot diameter at each measurement date for wild-type *Populus tremuloides* and crooked *P. tremuloides*. Diameter was measured at every second internode. Values indicated are least squares means (LS mean) and standard errors (SE) from a repeated measures ANOVA. (a) June 1, 2004; (b) June 7, 2004; (c) June 21, 2004; (d) July 5, 2004. * indicates significant difference ($P < 0.05$) in diameter at that particular internode between wild type and crooked aspen shoots (see Table 4).

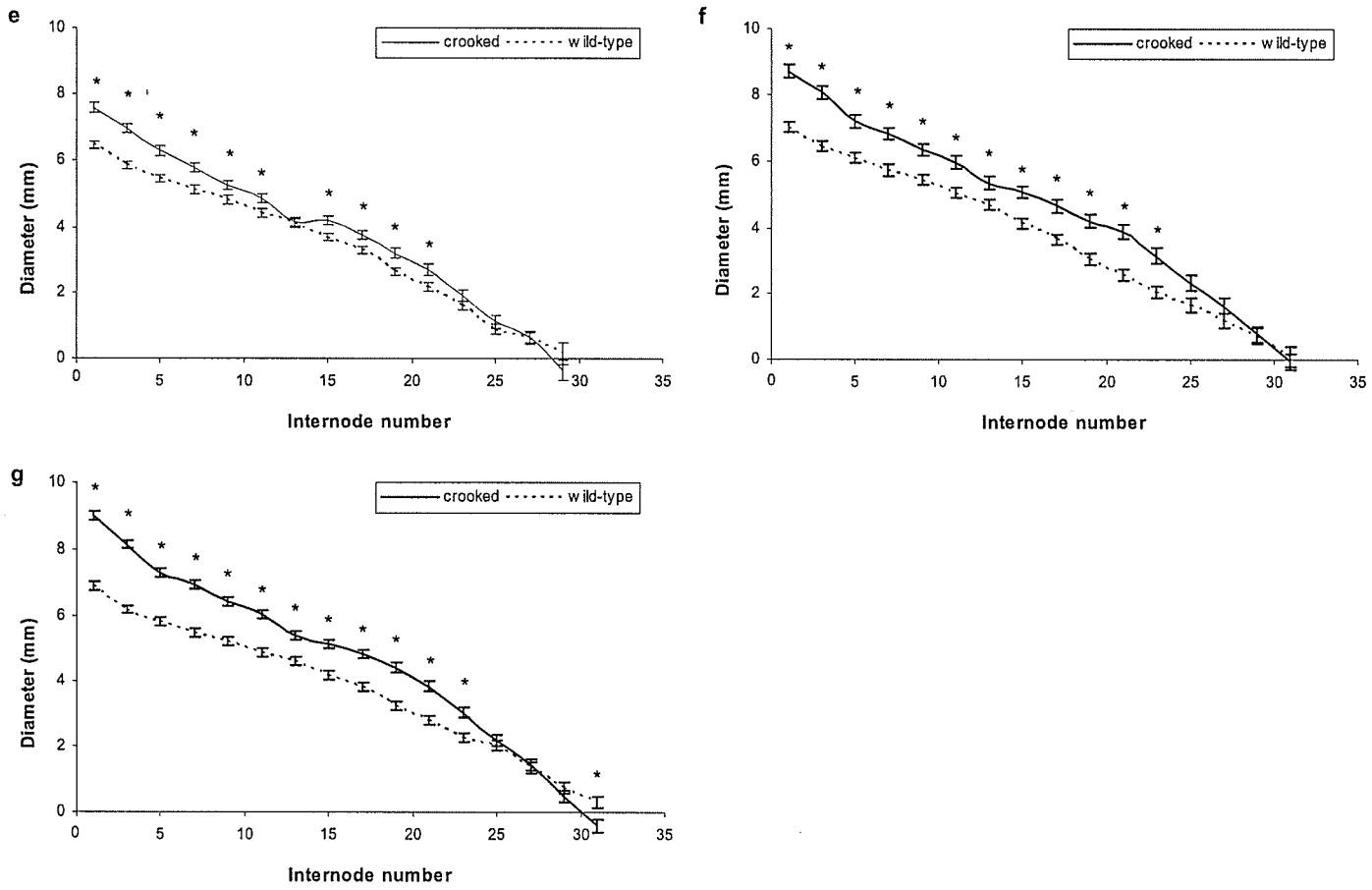



**Figure 11 *cont'd.*** (e) July 19, 2004; (f) August 3, 2004; (g) August 17, 2004. * indicates significant difference ($P < 0.05$) in diameter at that particular internode between wild-type and crooked aspen shoots (see Table 4).

tapered, as indicated by the negative slopes of plots for diameter vs. internode number. Significant differences between tree types occurred only in lower internodes early in the season. As the season progressed the differences for basal internodes increased and there was a significant difference between more of the internodes except those close to the tip.

### 4.2.5 Experimentally Manipulated Crooked Aspen Shoot Development

#### 4.2.5.1 Terminal Leaf Effects

Of the original 10 shoots that were subjected to terminal leaf removal only 6 developed into leading long shoots and therefore these were used for comparison with those of wild-type and crooked *P. tremuloides*. Shoot secant angle of termination was used to determine the effect of terminal leaf removal on crooked aspen shoot bending. As shown in section 4.2.2, wild-type aspen shoot secant angle remained relatively unchanged over the entire growing season, while crooked aspen shoot secant angle decreased as the shoots bent over the growing season. When the terminal leaves were removed, shoot secant angle changed little over the course of the growing season (Figure 12). Thus, unlike intact crooked aspen shoots, those with their terminal leaves removed did not bend down (in relation to gravity). It was noted that all other morphological variables were not noticeably different from non-manipulated crooked aspen shoots (data not shown).

#### 4.2.5.2 Gravimorphic Effects of Parent Shoot Reorientation

On crooked *P. tremuloides* leader shoots, the daughter shoots show a pattern of mesotony (Remphrey & Pearn 2003), whereby the longest lateral

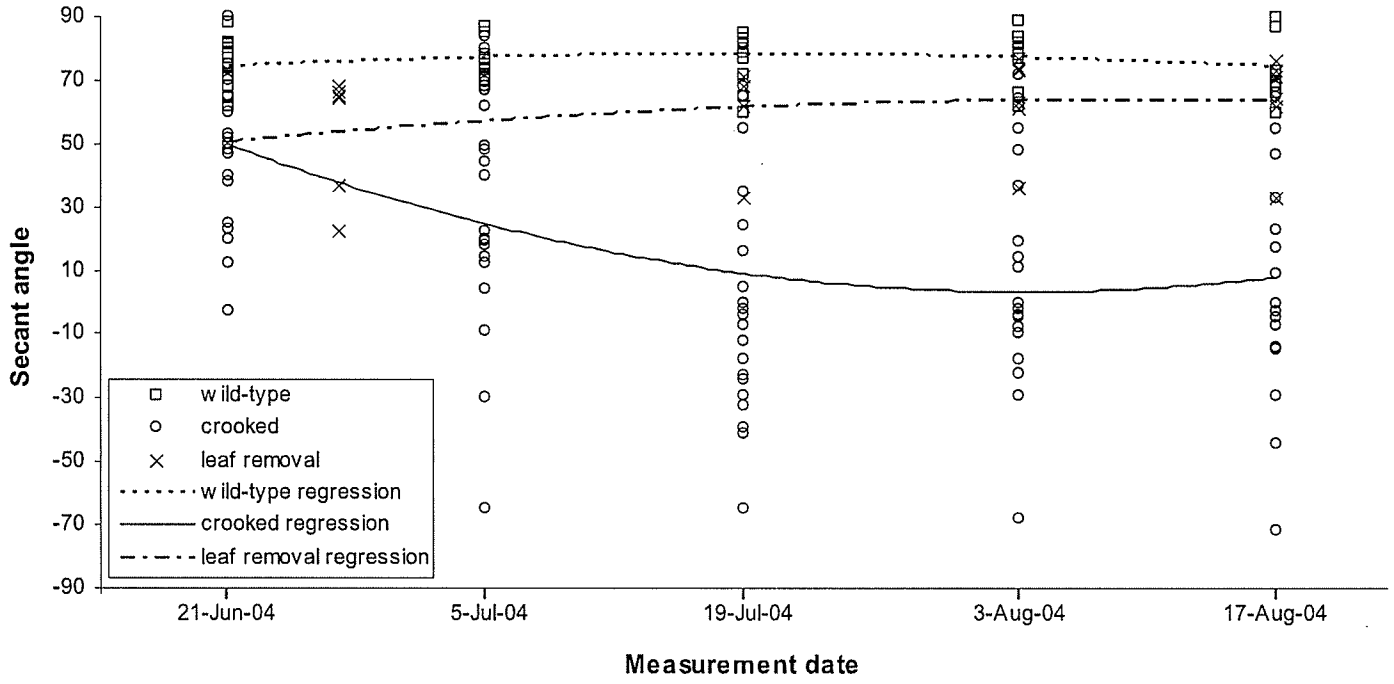



**Figure 12.** Change in secant angle (y) over time (x) comparing wild-type *Populus tremuloides* ($y = 69.30 + 5.90x - 0.98x^2$, $R^2 = 0.04$, n = 65), crooked *P. tremuloides* ($y = 84.60 - 39.87x + 4.90x^2$, $R^2 = 0.21$, n = 120), and crooked *P. tremuloides* shoots with terminal leaves removed ($y = 40.13 + 10.84x - 1.24x^2$, $R^2 = 0.07$, n = 24).

shoots arise from the central portion of the parent shoot. The pattern of daughter shoot development along vertically reoriented crooked aspen parent shoots was acrotonic, with the most vigorous daughter shoots arising at or near the parent shoot tip (Figure 13), typical of wild-type trembling aspen leader shoots (Jankiewicz & Stecki 1976; Isebrands & Nelson 1982). However, the emerging crooked aspen leader shoots bent in a similar fashion to leader shoots along unsuspended crooked parent shoots (T, Figure 13). Lateral shoots lower down along the vertically reoriented parent shoot also bent, becoming even more pendulous in orientation due to their initial elevation angles being nearly horizontal (no upwards growth) (D, Figure 13).

### 4.2.6 Shoot Strength and Elasticity

Prior to experimental shoot bending there was no significant difference in secant angle between the wild-type *Populus tremuloides* and crooked *P. tremuloides*, either in the field or in the lab (P = 0.90 and 0.72, respectively). There was also no significant difference in secant angle after weights were added (P = 0.17 and 0.08, respectively). In comparing the slopes of the change in shoot secant angle there was no significant difference between wild-type and crooked aspen shoots in either experiment (P = 0.23 and 0.31, respectively) (Figures 14 and 15). Based on an analysis of covariance (ANCOVA) there was no significant difference in the change in secant angle between tree types in either the field experiment or the lab experiment (P = 0.30 and 0.13, respectively).


**Figure 13.** Daughter shoot (D) development along a vertically reoriented crooked *Populus tremuloides* parent shoot (P), as seen August 6, 2003. Many daughter shoots are fully developed (one is marked D), along with a terminal leader daughter shoot (T). All daughter shoots are bent, exhibiting the crooked trait.

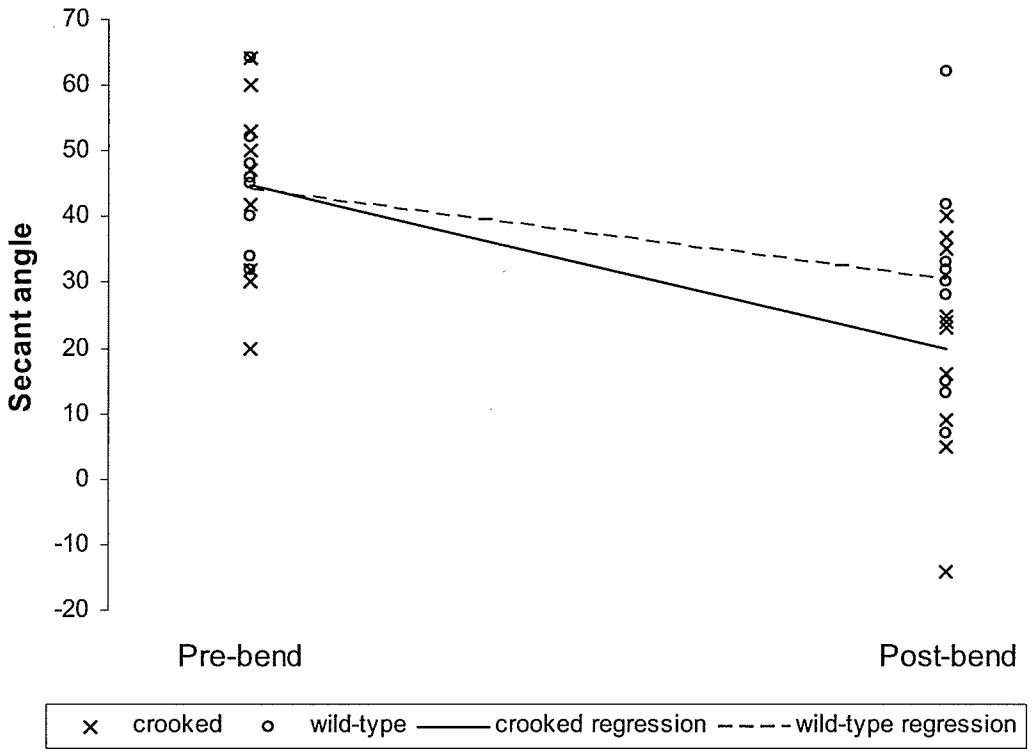



**Figure 14.** In-field shoot deflection analysis: change in secant angle of termination (y) measured before and after applying a weight (x) to inclined shoots of wild-type *Populus tremuloides* (broken line: $y = 57.8 - 13.7x$; $R^2 = 0.22$, $n = 20$) and crooked *P. tremuloides* (solid line: $y = 69.6 - 24.8x$; $R^2 = 0.42$, $n = 20$) prior to the time of shoot bending (June 29, 2004). Slopes were not significantly different ($P = 0.23$), and based on an analysis of covariance (ANCOVA) there was no significant difference in secant angle between tree types ($P = 0.30$).

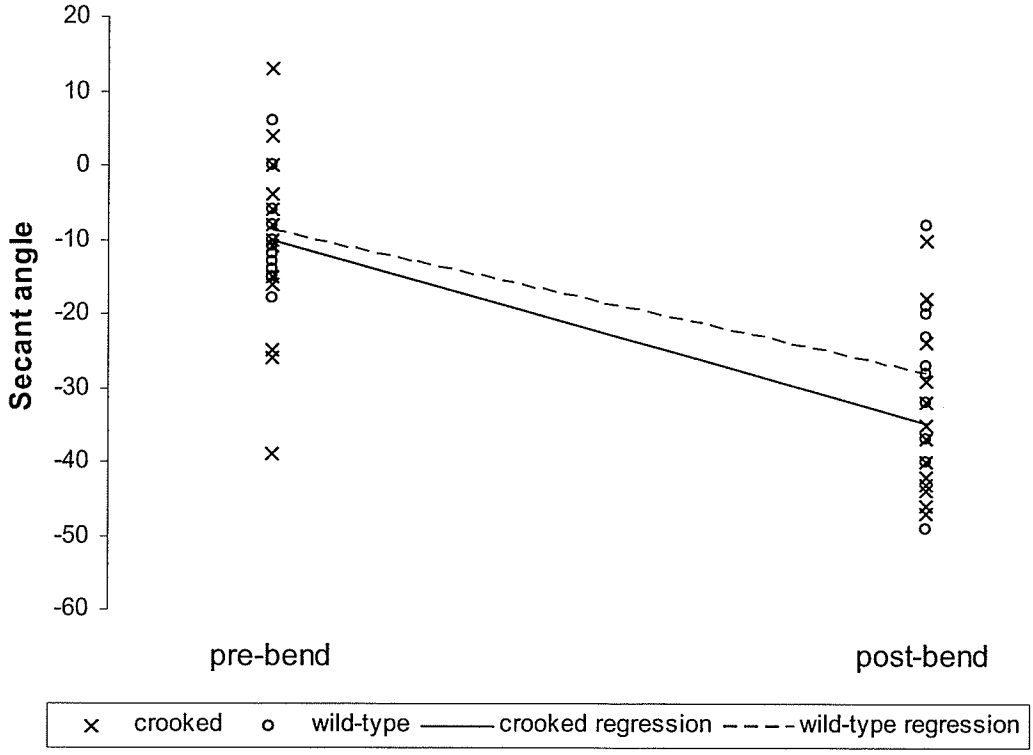



**Figure 15.** Laboratory shoot deflection analysis: change in secant angle of termination (y) measured before and after applying a weight (x) to horizontally oriented shoots of wild-type *Populus tremuloides* (broken line: $y = 10.4 - 19.27x$; $R^2 = 0.60$, $n = 30$) and crooked *P. tremuloides* (solid line: $y = 14.47 - 24.67x$; $R^2 = 0.53$, $n = 30$) prior to the time of shoot bending (June 29, 2004). Slopes were not significantly different ($P = 0.31$), and based on an analysis of covariance (ANCOVA) there was no significant difference in secant angle between tree types ($P = 0.13$).

The initial displacement from horizontal, due to self weight, of experimentally harvested shoots was similar between wild-type and crooked aspen shoots (t-test: $P = 0.45$); however, after applying a weight to the horizontally oriented shoots there was a significant difference in terminal end displacement ($P < 0.01$). Crooked aspen shoots had greater mean (± SE) displacement (-20.8 ± 1.73 cm) compared to wild-type aspen shoots (-14.9 ± 1.11 cm) after applying the weight. However, the slope of the change in displacement was not significantly different ($P = 0.18$) between wild-type and crooked aspen shoots (Figure 16).

### 4.3 Shoot Ontogeny: Anatomical Analysis

#### 4.3.1 Radial Growth and Shoot Symmetry

Anatomical analyses revealed that sections were similar among all observed samples of each experimental shoot type. As radial growth in *Populus tremuloides* shoots increased, the proportion of each major tissue type varied among different tree types (Figure 17). Initially, all shoots had relatively large piths with proportionally less xylem, phloem, and cortex tissue development (Figure 18). With the initiation of secondary growth, the amount of each observed tissue type increased, but at different rates. There was a very slight increase in cortex and phloem tissues over time, and a much greater rate of increase in xylem tissue (Figure 17). On the final measurement date (July 20, 2004) there was an observed decrease in pith size in several shoots concomitant with increased xylem production.

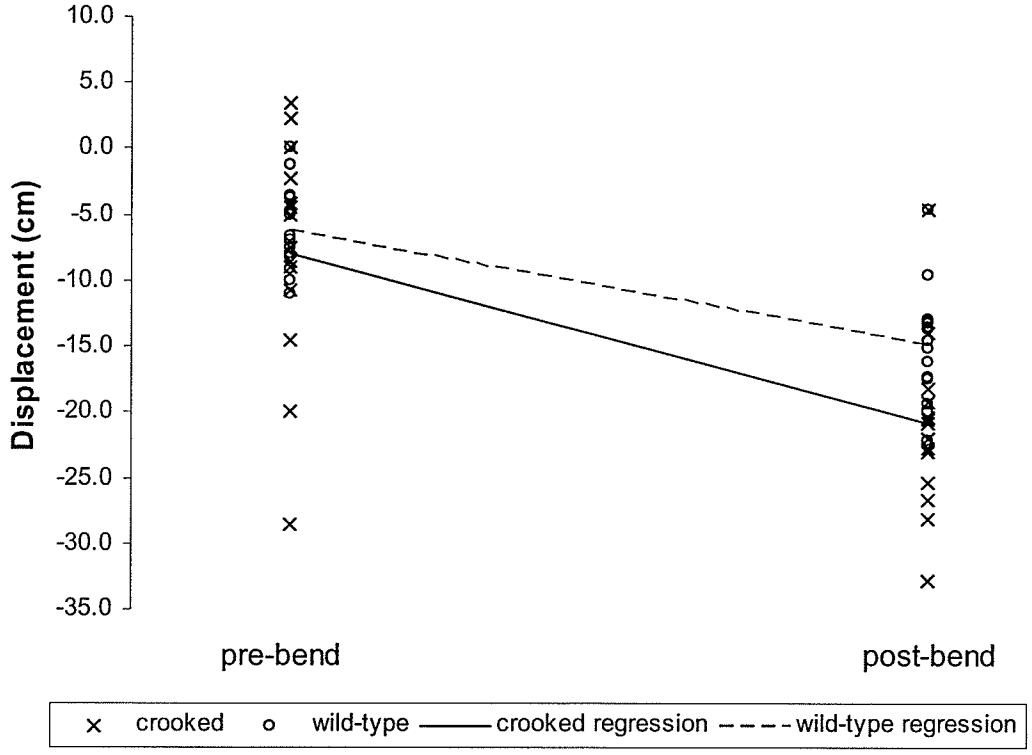



**Figure 16.** Laboratory shoot deflection analysis: displacement distance of the terminal end from horizontal (y) measured before and after applying a weight (x) to horizontally oriented shoots of wild-type *Populus tremuloides* (broken line: $y = 2.42 - 8.64x$; $R^2 = 0.58$, $n = 30$) and crooked *P. tremuloides* (solid line: $y = 4.83 - 12.83x$; $R^2 = 0.43$, $n = 30$) prior to the time of shoot bending (June 29, 2004). Before experimental bending t-tests revealed no difference between tree types ($P = 0.45$); however, after bending the displacement was greater in crooked aspen shoots ($P < 0.01$). Slopes were not significantly different ($P = 0.18$).

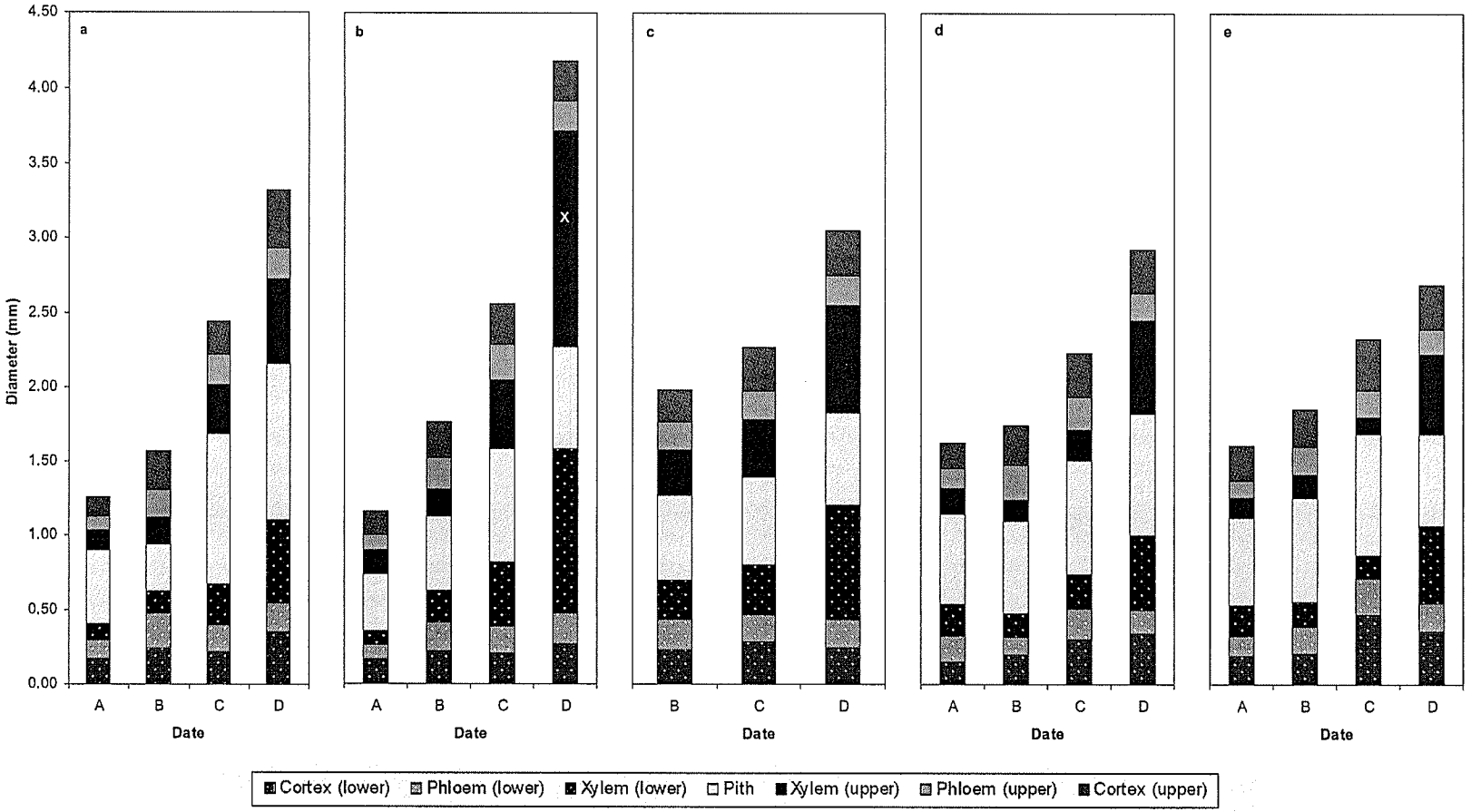



**Figure 17.** Relative amount of each major tissue type in sampled *Populus tremuloides* shoots. Tissues were measured from one side to the opposite in vertically oriented shoots, and from the upper side to the lower side of bent shoots. Wild-type aspen shoots (a), crooked aspen shoots (b), vertically trained crooked aspen shoots (c), wild-type aspen shoots oriented horizontally (at 0°) (d), and wild-type aspen shoots oriented at 45° (e) were sampled June 8, 2004 (A), June 22, 2004 (B), July 6, 2004 (C), and July 20, 2004 (D). X indicates the location of shoot asymmetry (P = 0.02), with more xylem found on the upper side of the shoot.

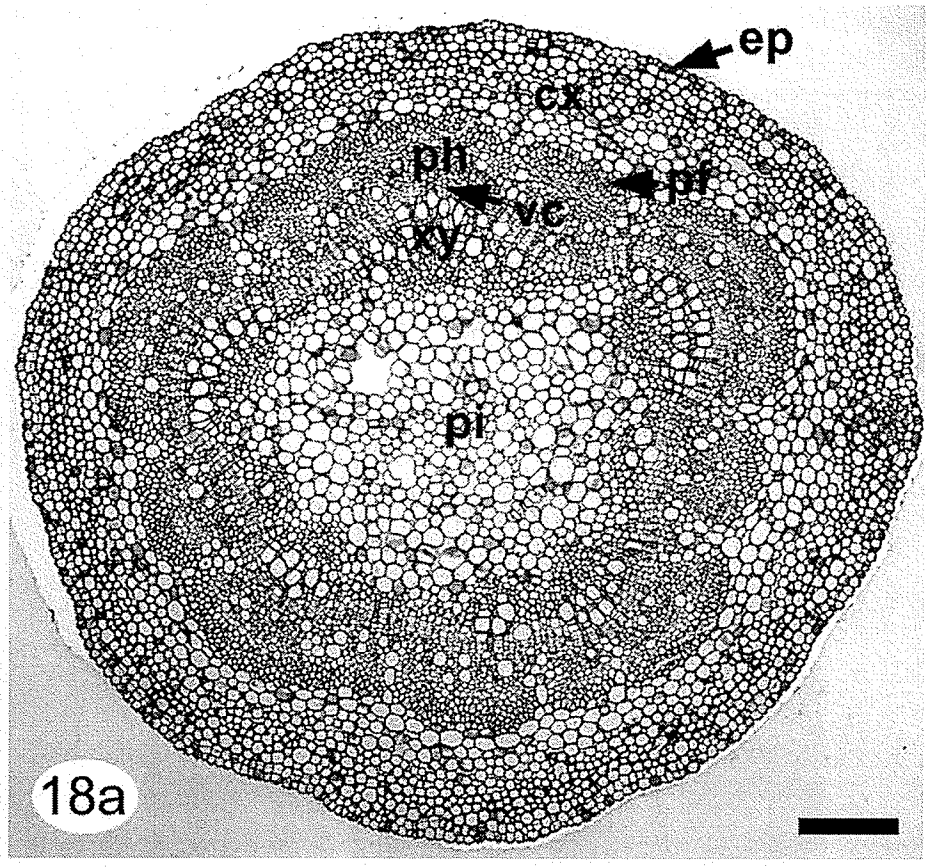
On the final measurement date an analysis of variance revealed that bent crooked aspen shoots had significantly more xylem tissue ($P < 0.01$) than any other tree/shoot type. Also, crooked aspen shoots had significantly less pith than wild-type aspen shoots ($P = 0.02$). There was no significant difference in the amount of other tissue types between each observed tree/shoot type on the final measurement date.

Shoot asymmetry was observed in bent crooked aspen shoots on the final measurement date, with more xylem tissue formed on the upper side compared to the lower side of bent shoots ($P = 0.02$) (Figure 17). There was no significant asymmetry in other tissue types within any other tree/shoot type.

### 4.3.2 Developmental Shoot Anatomy

Sampled wild-type aspen shoots oriented at different angles off vertical were similar in anatomy to the terminal leader wild-type aspen shoots and are therefore not presented in this section. Early in development relatively few structural tissues existed, and those that were present were poorly developed. On the first sample date (June 8, 2004) the vascular cambium had initiated secondary growth, producing secondary xylem to the inside and secondary phloem to the outside (Figures 18, 19a and b). The secondary xylem was made up of numerous vessel elements for water conduction, with proportionally fewer structural fibres. A layer of collenchyma tissue was observed within the cortex, beneath the epidermis. Primary phloem fibres were present as large bundles of sclerenchyma cells with lignified cell walls (Figures 19c and d). On the second measurement date (June 22, 2004), approximately one week prior to crooked

**Figure 18.** Light micrographs of transverse sections of whole shoots of wild type (a) and crooked (b) aspen early in development (June 8, 2004). The relatively large central pith (pi) is surrounded by vascular tissues including xylem (xy) and phloem (ph). The vascular cambium (vc) has been initiated, producing secondary xylem inwards and secondary phloem outwards. Phloem fibres (pf) are apparent as large bundles almost forming a complete cylinder of structural tissue in the young shoot. A layer of collenchyma tissue is found in the outermost cortex (cx), just beneath the epidermis (ep). Stained with periodic acid-Schiff – toluidine blue O (PAS – TBO). Bar = 157 µm.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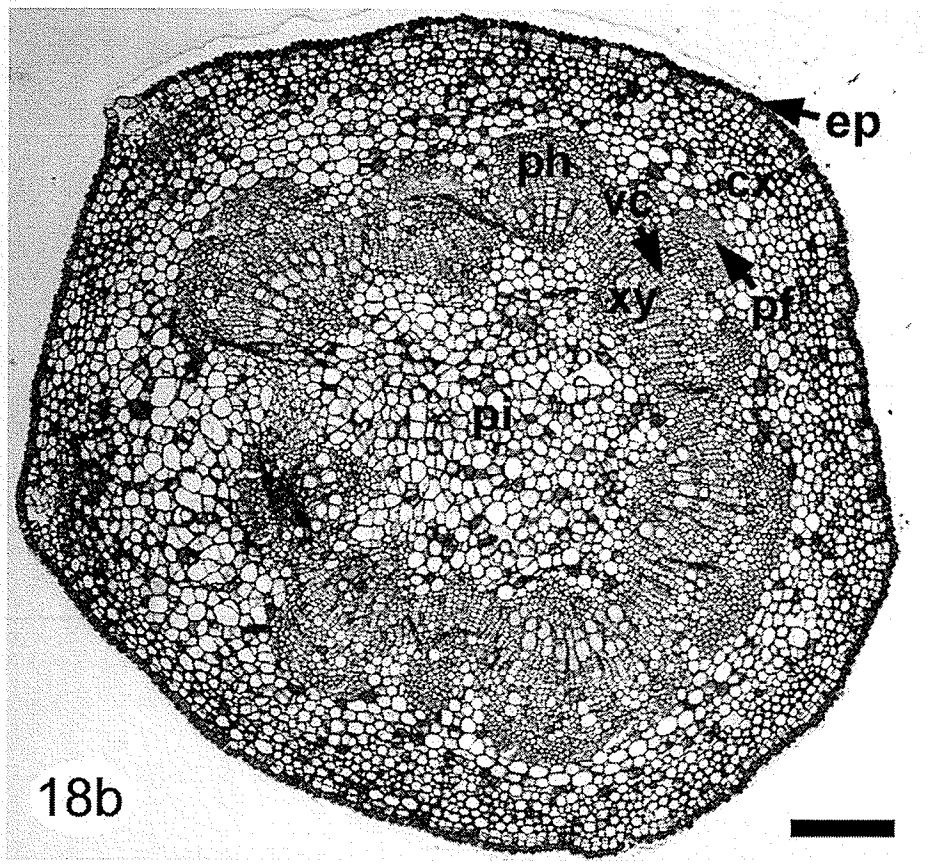
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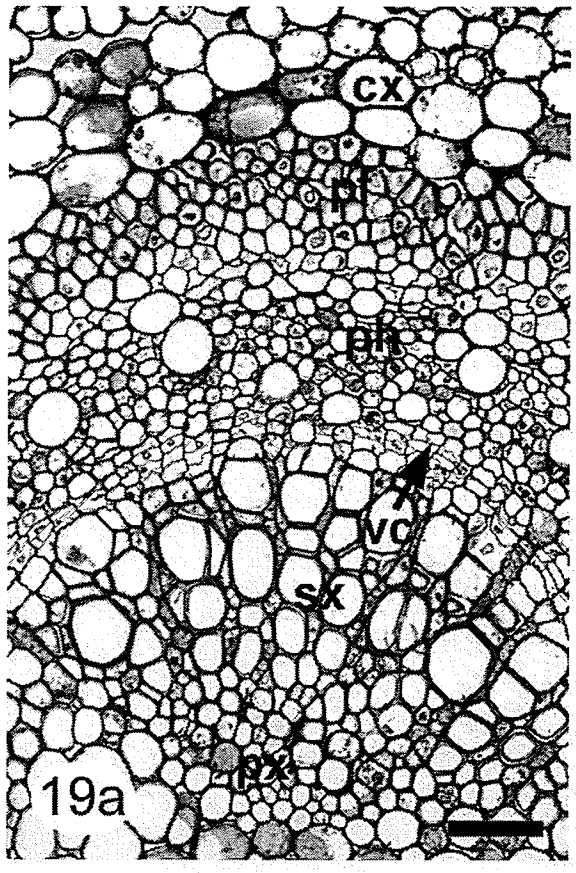
**Figure 19.** Increasingly higher magnification light micrographs of portions of transverse sections of wild-type (a and c) and crooked (b and d) aspen shoots on June 8, 2004. Primary (px) and secondary (sx) xylem tissues are apparent in the wild-type section (a). Phloem fibres (pf) are composed of sclerenchyma cells having thick cell walls (cw) compared to the thin-walled parenchyma cells of the cortex (cx). At this stage of development the sclerenchyma cells maintain a relatively large lumen (lu) in the center of the cell. vc, vascular cambium; ph, phloem tissue; co, collenchyma; ep, epidermis. Stained with PAS – TBO. Bar = 40 µm (Figures 19a and b), and 20 µm (Figures 19c and d).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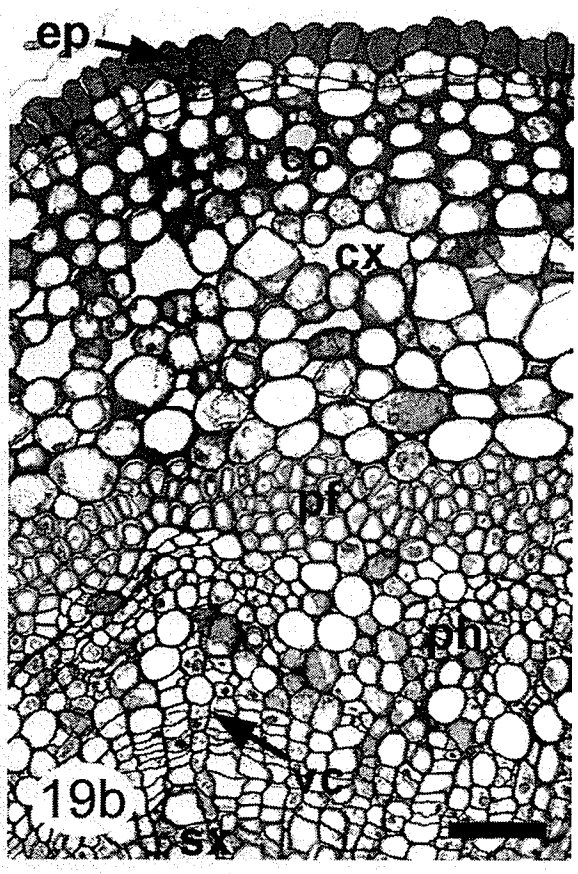
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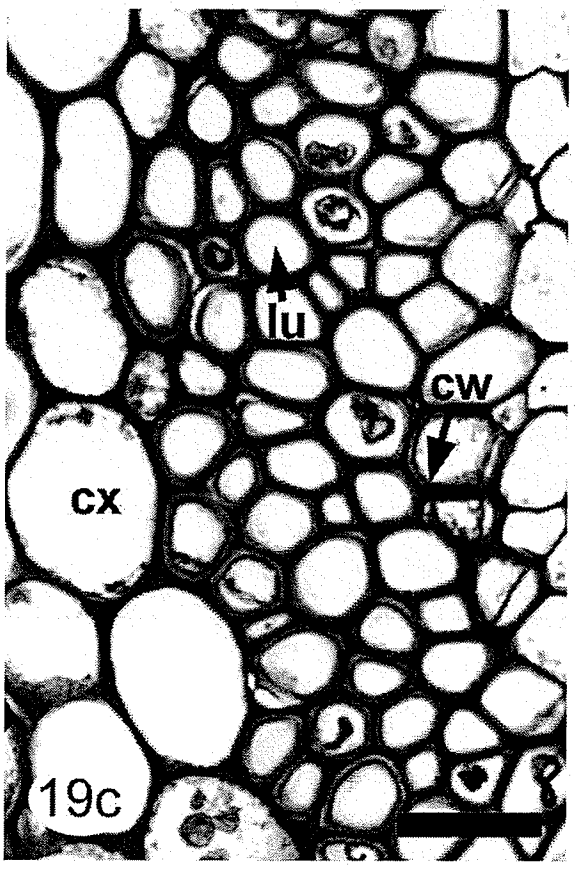
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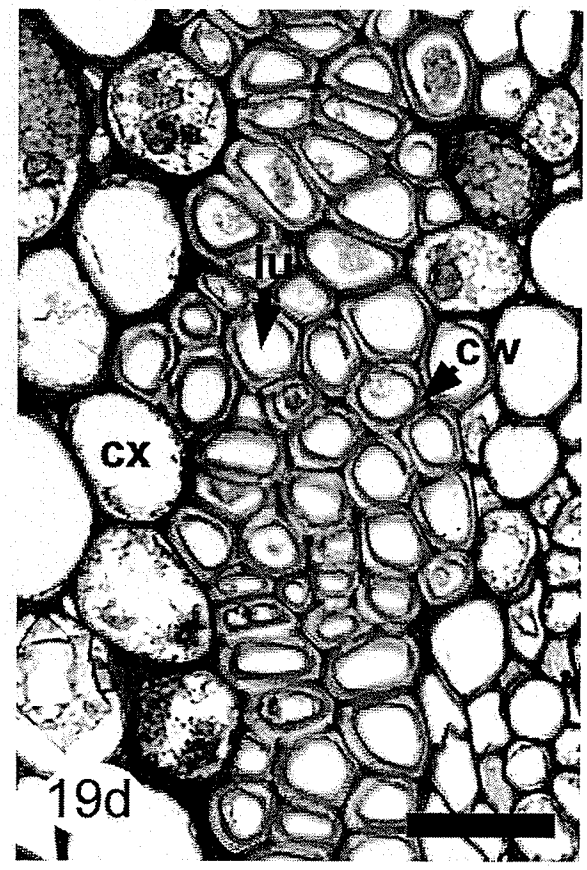
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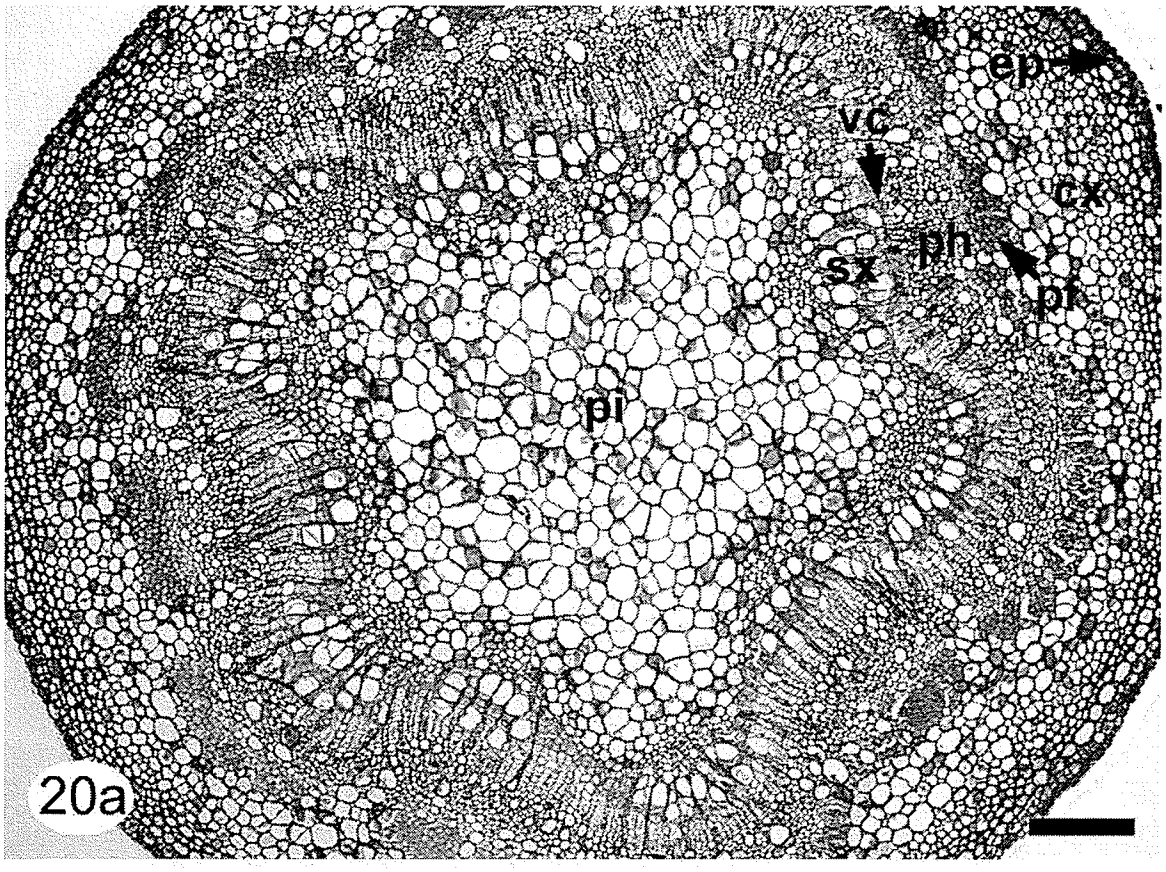
aspen shoot bending, both wild-type and crooked aspen shoots showed a similar increase in secondary xylem tissue (Figures 20, 21a and b). Primary phloem fibres showed increased cell wall lignification in all tree/shoot types (Figures 21c and d).

Following the time of crooked aspen shoot bending several differences in structural shoot anatomy were observed from the wild type. On the third sample date (July 6, 2004) the phloem fibre cell walls of wild-type aspen shoots showed increased lignification, with smaller central lumens than previously observed (Figures 22a and b). Both bending and vertically trained crooked aspen shoots showed lateral differences in phloem fibre lignification on this date. One side (lower side in bending shoots) had nearly fully lignified cells, while the opposite side (upper side in bending shoots) maintained a lumen similar to that of wild-type aspen phloem fibres (Figures 22c, d, e, and f). On the final sample date (July 20, 2004) wild-type phloem fibres were fully lignified (Figures 23a), while bent and vertically trained crooked aspen shoots maintained the same lateral difference in phloem fibre lignification observed on the third measurement date (Figures 23b, c, d, and e).

By the third and fourth measurement date (July 6, 2004 and July 20, 2004, respectively), as more xylem tissue formed, there was a greater proportion of xylem fibres to vessel elements (Figures 24 and 25). Also, the gelatinous layer (G-layer), characteristic of tension wood, was first identified within fibre cells on the lower side of crooked aspen shoots following the initiation of shoot bending, July 6, 2004 (Figure 24d). At this stage of development the G-layer had only

**Figure 20.** Light micrographs of transverse sections of wild-type (a) and crooked (b) aspen shoots with relatively vertical orientation, just prior to the approximate time of crooked shoot bending (June 22, 2004). Shoots are relatively symmetric, with erratic piths (pi), and evidence of increased secondary xylem (sx) production. Only slightly more secondary phloem tissue (ph) was produced. Phloem fibres (pf) are found in large discrete bundles, and are more heavily stained blue signifying increased lignification. Note: the stiffness of the phloem fibres (pf) is revealed by the inability of the microtome knife to cut evenly through the tissue, causing them to separate. vc, vascular cambium; cx, cortex; ep, epidermis. Stained with PAS – TBO. Bar = 157 µm.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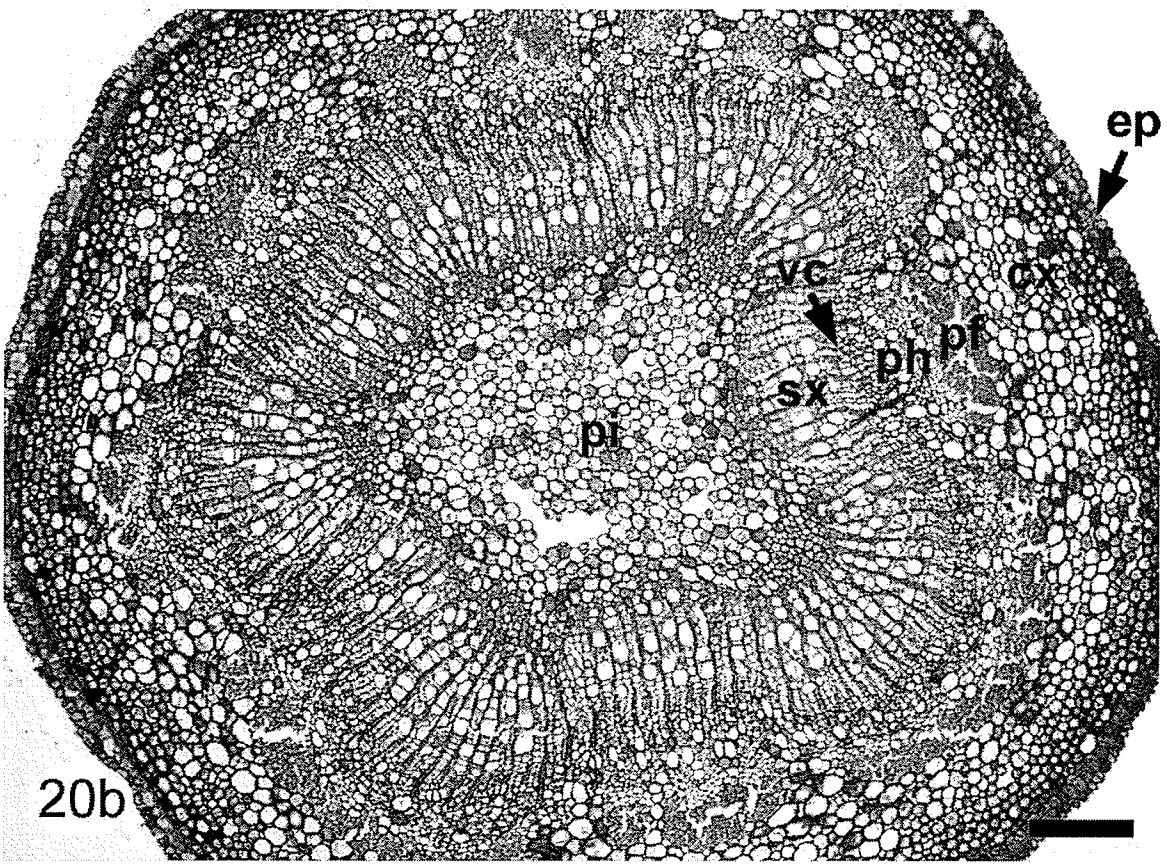
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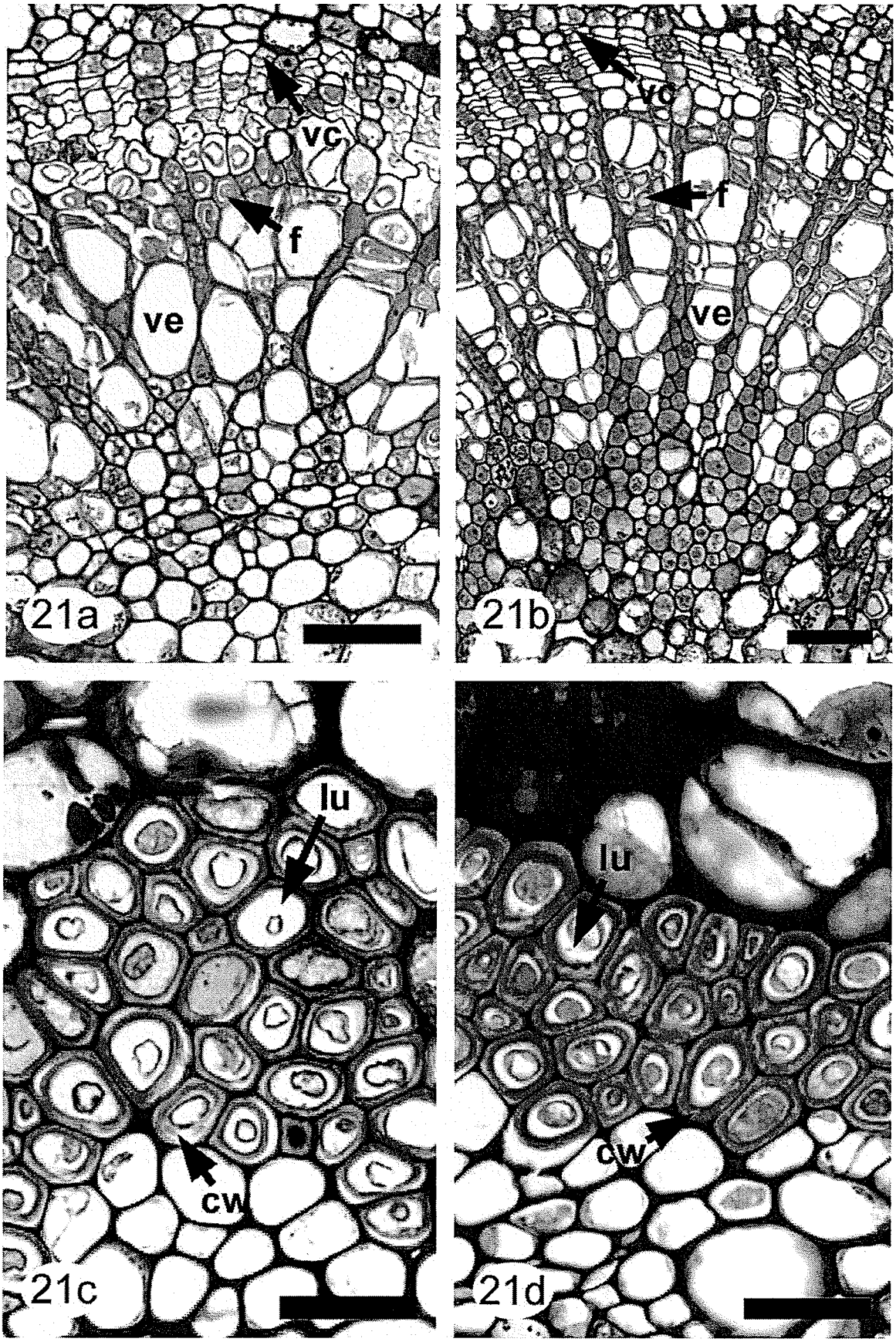
**Figure 21.** Increasingly higher magnification light micrographs of portions of transverse sections of wild-type (a and c) and crooked (b and d) aspen shoots on June 22, 2004. Numerous fibres (f) are present in the secondary xylem. Relatively few vessel elements (ve) are found in these sections. Sclerenchyma cells of phloem fibres have thick, lignified cell walls (cw), but maintain a central lumen (lu). vc, vascular cambium. Stained with PAS – TBO. Bar = 40 µm (Figures 21a and b), and 20 µm (Figures 21c and d).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21b
lu
cw
21c
lu
cw
21d

**Figure 22.** Light micrographs of transverse sections of primary phloem fibres of wild-type aspen (a and b), crooked aspen (c and d), and vertically trained crooked aspen (e and f) shoots sampled after crooked shoot bending (July 6, 2004). Each pair of micrographs (a and b, c and d, e and f) represents phloem fibres from opposite sides of shoots (upper vs. lower in bent crooked aspen shoots). Wild-type shoots maintained a central lumen in the fibres on either side of the shoot (a and b), while only fibres found on the upper side of crooked aspen shoots (c) had a large lumen. Fibres on the lower side of the shoot (d) were nearly fully lignified. Vertically trained crooked aspen shoots showed a similar pattern of phloem fibre lignification as bent crooked aspen shoots, that is cells with large lumens were found on one side of the shoot (e) and fully lignified cells were found on the opposite side of the shoot (f). Arrows mark central lumens within phloem fibre sclerenchyma cells. Stained with PAS – TBO. Bar = 20 µm.

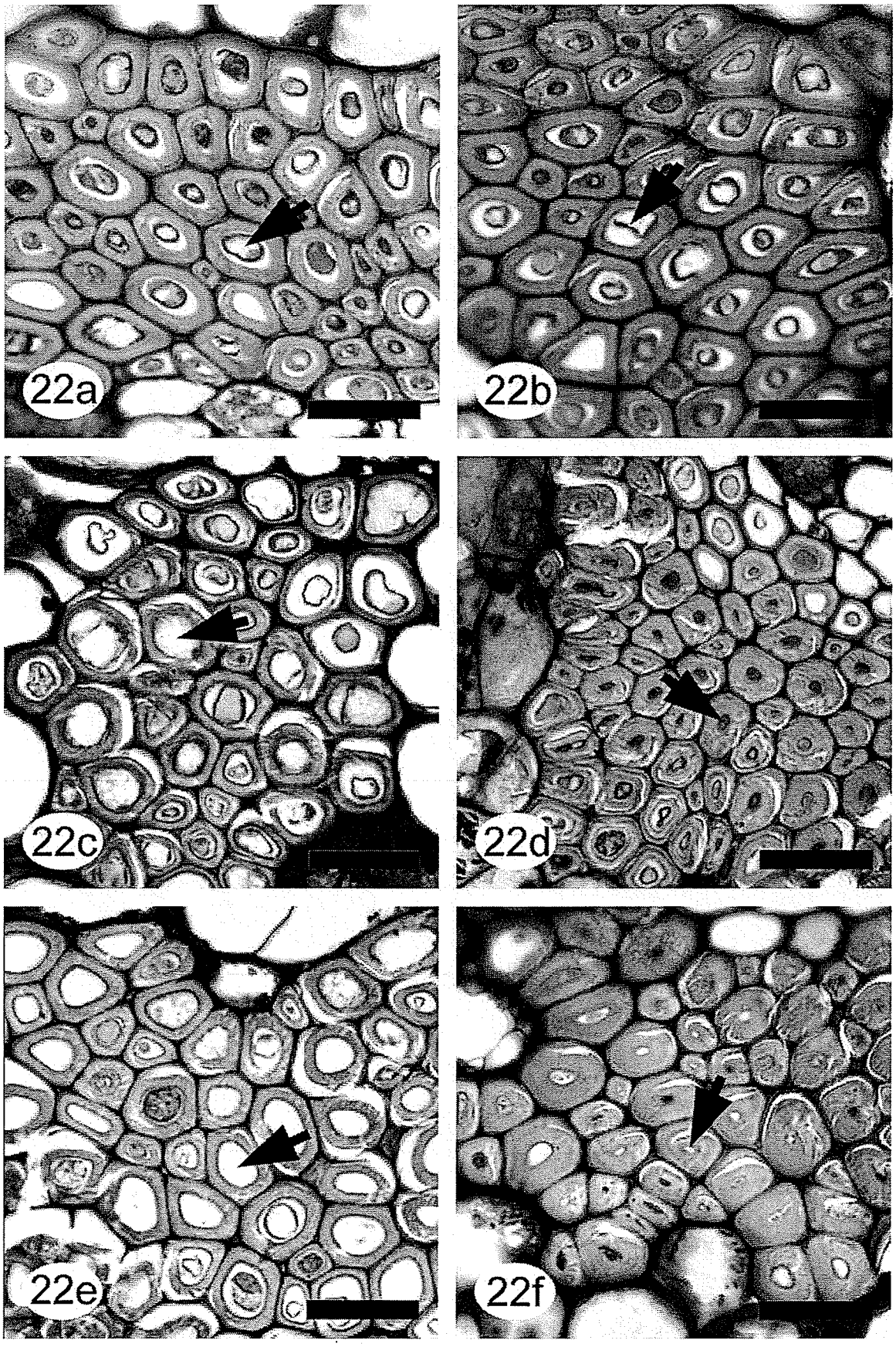
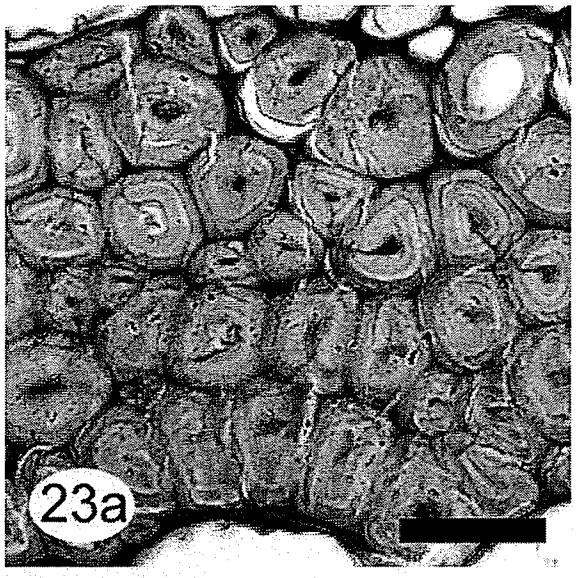
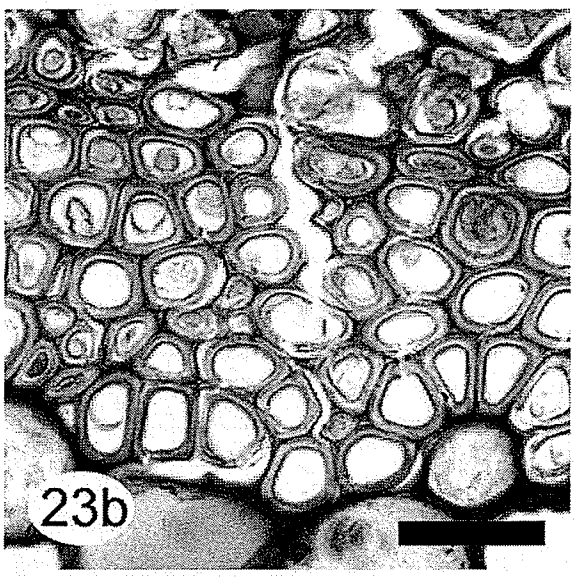
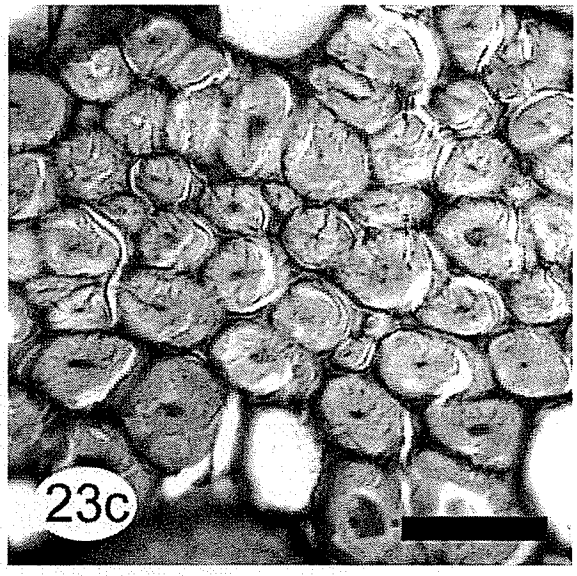
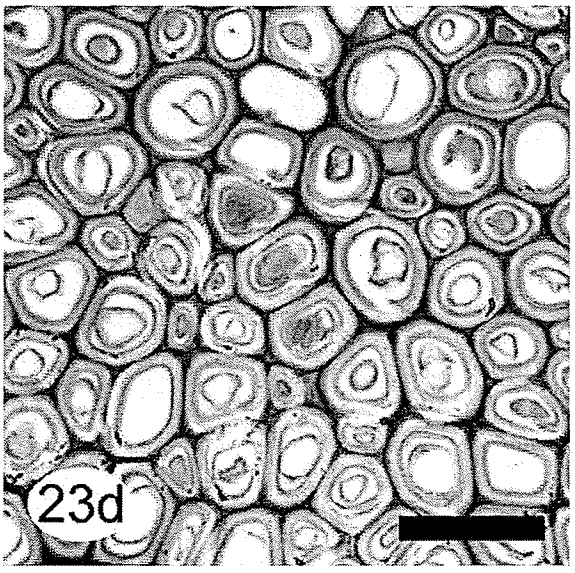

22a
22b
22c
22d
22e
22f

**Figure 23.** Light micrographs of transverse sections of primary phloem fibres of wild-type aspen (a), crooked aspen (b and c), and vertically trained crooked aspen (d and e) shoots sampled later in the growing season (July 20, 2004). The individual micrograph (a) is representative of all fibres in those sections. Each pair of micrographs (b and c, d and e) represents phloem fibres from opposite sides of shoots (upper vs. lower in bent crooked aspen shoots). Cells within all fibres of wild-type shoots (a) were fully lignified, while only fibres found on the lower side of crooked aspen shoots (c) were fully lignified. Cells within fibres on the upper side of crooked aspen shoots (b) maintained a central lumen. Vertically trained crooked aspen shoots showed a similar pattern of phloem fibre lignification to bent crooked aspen shoots, cells with large lumens found on one side of the shoot (d) and fully lignified cells found on the opposite side of the shoot (e). Stained with PAS – TBO. Bar = 20 µm.


23a


23b


23c


23d

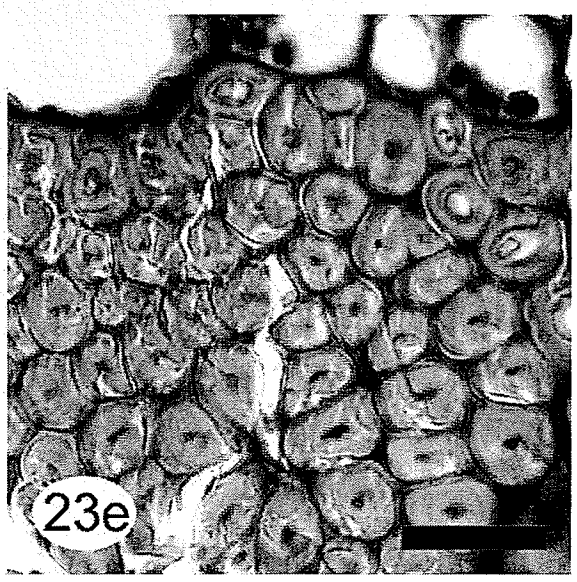

23e

**Figure 24.** Light micrographs of transverse sections of secondary xylem tissue of aspen after the start of crooked aspen shoot bending (July 6, 2004). Wild-type aspen (a) and vertically trained crooked aspen xylem (b) was uniform in anatomy all around the shoot. Crooked aspen xylem anatomy differed between the upper (c) and lower (d) sides of shoots. A magnified view of secondary xylem tissue reveals numerous fibres (f) among larger vessel elements (ve). Sections were stained to show the presence or absence of tension wood. Lignified cell walls stain pink, while the gelatinous layer (G-layer) stains blue, and can be seen in gelatinous fibres (gf) on the lower side of bent crooked aspen shoots (arrows in d). Stained with safranin O – astra blue. Bar = 20 µm.

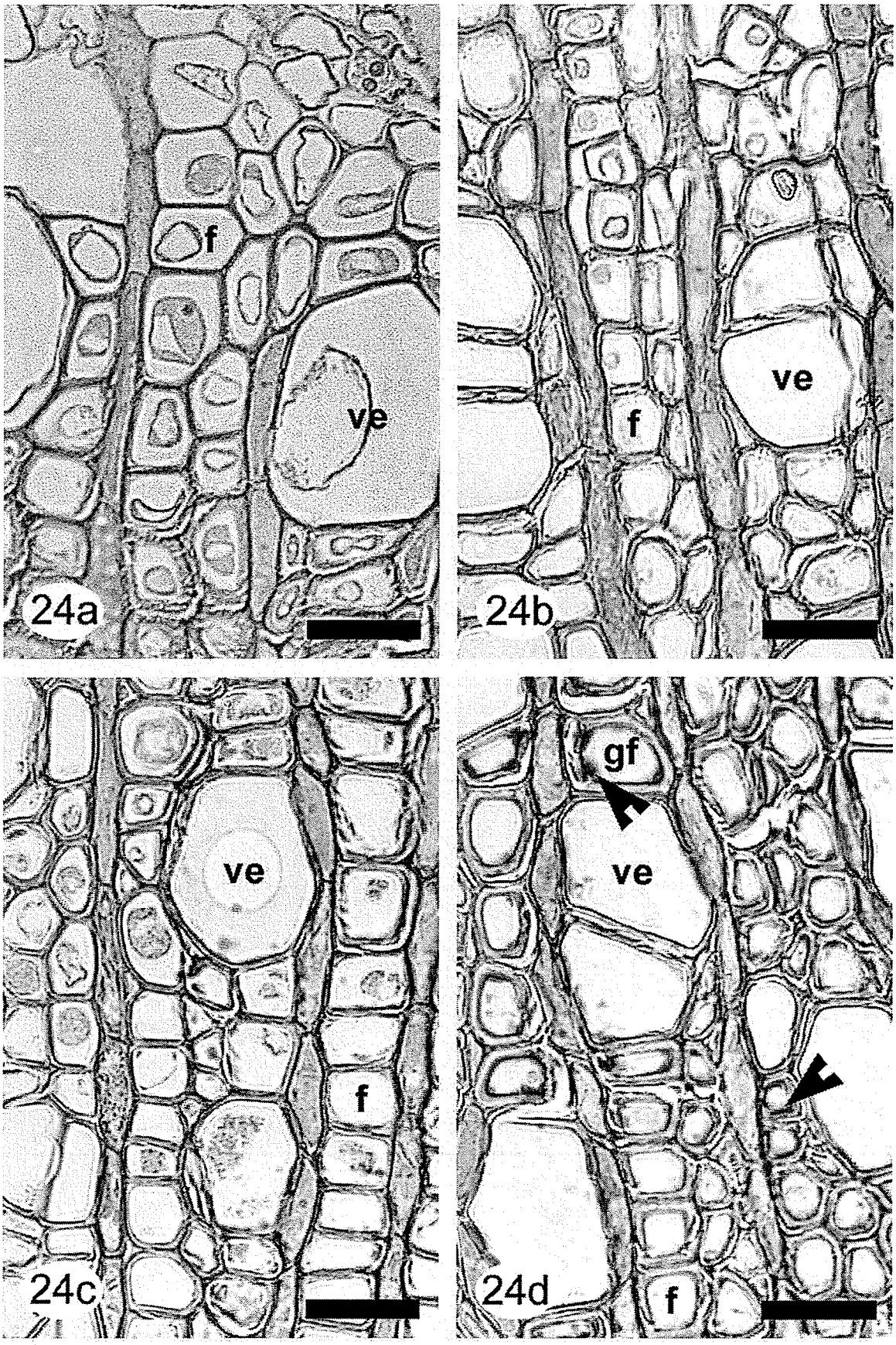
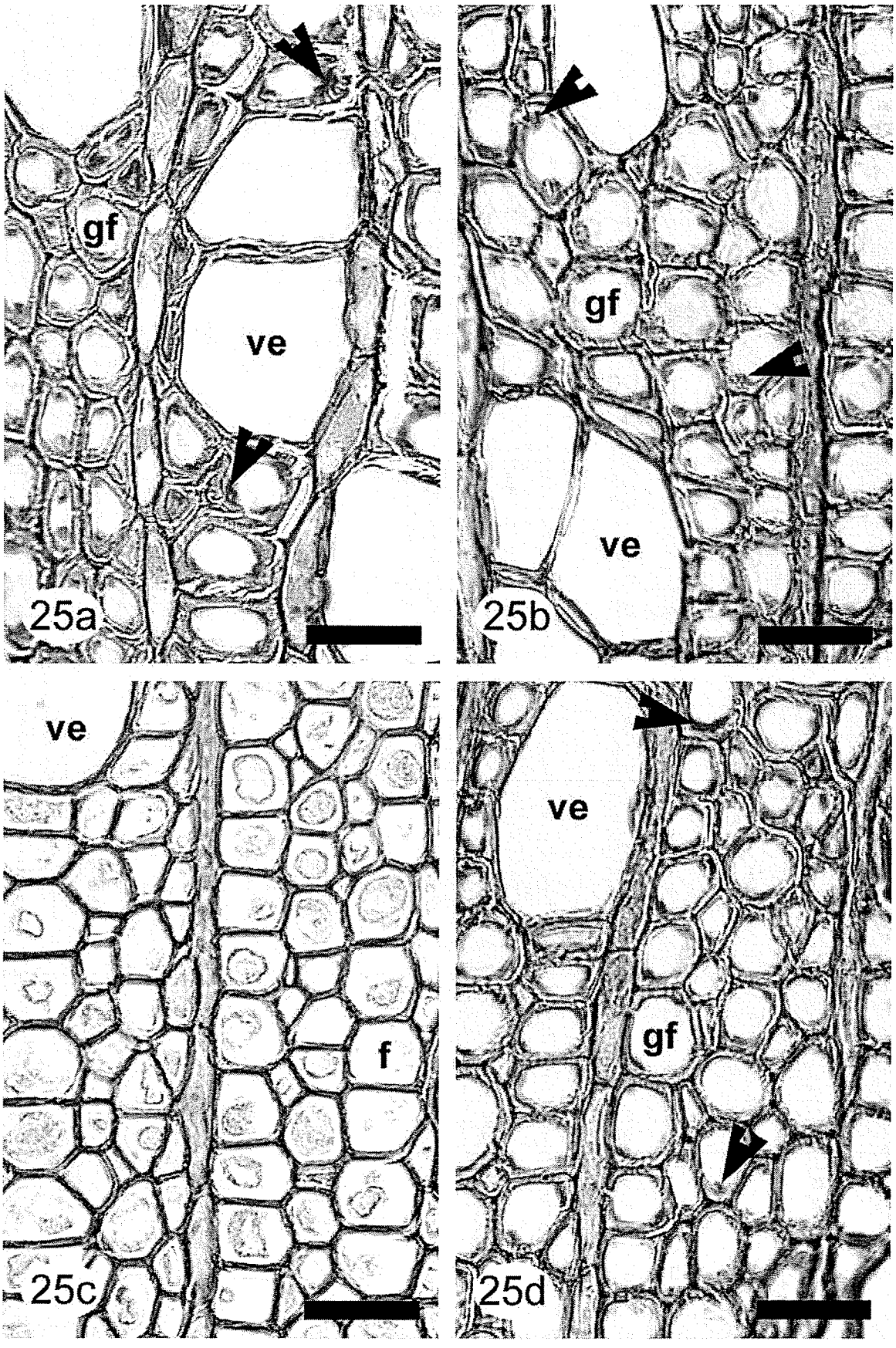

f
ve
24a
ve
f
24b
ve
f
24c
gf
ve
f
24d

**Figure 25.** Light micrographs of transverse sections of secondary xylem tissue sampled later in the growing season (July 20, 2004). Wild-type aspen (a) and vertically trained crooked aspen xylem (b) was uniform in anatomy all around the shoot. Crooked aspen xylem anatomy differed between the upper (c) and lower (d) sides of shoots. Sections were stained to show the presence or absence of tension wood. On this date gelatinous layers were observed throughout sections of wild-type aspen and vertically trained crooked aspen shoots (arrows in a and b). The lower side of bent crooked aspen shoots (d) also showed evidence of gelatinous layers (arrows), however, the upper side of crooked aspen shoots (c) was void of such gelatinous layers as was observed in Figure 24. f, fibres; gf, gelatinous fibres; ve, vessel elements. Stained with safranin O – astra blue. Bar = 20 µm.


gf
ve
25a
gf
ve
25b
ve
f
25c
ve
gf
25d

formed in the corners of the cells. Wild-type aspen, vertically trained crooked aspen, and the upper side of bending crooked aspen shoots did not show evidence of the G-layer on the third sample date (Figures 24a, b, and c). However, on the fourth sample date (July 20, 2004) gelatinous fibres (with associated G-layers) were identified throughout the xylem tissue of wild-type aspen and vertically trained aspen shoots (Figures 25a and b). Conversely, on this date G-fibres were still only found within xylem tissue on the lower side of bent crooked aspen shoots (Figure 25d), the upper, asymmetric sides having relatively thin fibre cell walls and no G-layers (Figure 25c).

## 4.4 Observations of Growth

### 4.4.1 Estimation of Young's Modulus

Initially, there was no significant difference in shoot fresh weight between wild-type and crooked aspen shoots ($P = 0.39$). Following oven drying the mean moisture content was calculated and crooked aspen shoots were found to have significantly greater percent moisture content than wild-type aspen shoots (Table 5). The mean values for moisture content (93.45% and 115.02%) fell within the range of 60-150% used by Cannell and Morgan (1987) and Young's modulus ($E$) was therefore calculated for the aspen shoots from the regression equation. $E$ was significantly greater in wild-type aspen shoots compared to crooked aspen shoots (Table 5).

**Table 5.** Comparison of mean variables involved in shoot elasticity measurements between wild-type (n = 12) and crooked (n = 12) *Populus tremuloides* shoots.

| Variable | Wild-type [a] | Crooked [a] | P > \| t \| [b] |
|---|---|---|---|
| Moisture content (%) | 93.45 ± 1.14 | 115.02 ± 2.06 | < 0.01 |
| Young's modulus (*E*) | 3.73 ± 0.05 | 2.76 ± 0.09 | < 0.01 |

[a] Values are means ± standard error (SE).
[b] Significance levels (P) based on paired t-tests.

# 5.0 DISCUSSION

The study of shoot development and branching patterns in woody plants provides a detailed understanding of the overall architecture. However, anatomical studies are essential to fully understand the structure and function of associated organs. Examining deviations from normal developmental patterns, as seen in mutant plants, can provide insight to the mechanisms involved in creating these patterns in all plants (Myers et al. 1994; Digby & Firn 1995).

Early in the growing season wild-type and crooked aspen shoots were morphologically alike, developing with similar shape and size. At this stage of growth the aspen shoot is primarily functioning in water transport (Dickmann et al. 2001), as was reflected in the observed xylem tissue containing numerous vessel elements (Bowes 1996; Jourez et al. 2001). With increased water transport comes increased turgor pressure, which would have provided rigidity to the young, relatively small shoots (Zimmermann & Brown 1971; Salisbury & Ross 1992), and help maintain a relatively vertical orientation, as was observed in both wild-type and crooked aspen trees. This orientation was maintained despite the absence of significant amounts of structural tissues (Mauseth 1988), suggesting that, because these shoots are small and light, they can support their own weight. There were some support tissues found in young aspen shoots. These included a band of collenchyma tissue along the periphery of all shoots, known as a primary support tissue, providing plastic support and structure to the shoot, while allowing for extension growth (Mauseth 1988; Bowes 1996; Dickison 2000),

and partly lignified cell walls in primary phloem fibres that provide some structural support to the shoot (Den Outer 1993; Niklas 1999; Chaffey et al. 2000; Quilhó et al. 2000).

As shoot expansion progressed, the orientation of crooked aspen shoots became slightly oblique compared to wild-type shoots. It was unclear what factors caused shoot leaning, as the shoots of both tree types were similar in all other obvious aspects of morphology and anatomy. Crooked aspen shoot leaning resembles, and might be attributed to, the inability to support itself due to poor strength. Whatever the cause, leaning appears to be the initial stage of shoot bending, and provides a cue for subsequent morphological and anatomical changes observed in relation to the gravity stimulus. Ultimately, shoot leaning distinguishes upper from lower sides in relation to gravity, and this is known to subsequently influence the distribution of certain hormones that are involved in shoot development, including auxin (Cline 1991; Wilson 2000). These hormones are known to accumulate on the lower side of the stem where they can elicit the formation of specialized tissues (Wareing & Nasr 1961; Wilson & Archer 1977).

Following the onset of shoot leaning, midway through the growing season, a fairly rapid bending motion was observed in crooked aspen shoots over a relatively short period of time. The major point of bending was observed in several consecutive internodes formed approximately midway through the growing season. Initial bending of these internodes was somewhat removed from the tip. At the same time, wild-type leader shoots maintained a relatively straight, vertical orientation. Shoot bending was observed as a sharp decrease

in shoot secant angle of termination, while the basal angle of elevation remained relatively unchanged. As a result, bent crooked aspen shoots maintained a relatively upright basal portion, on average approximately one quarter the final length of the shoot, followed by a shorter bending point becoming horizontal, and a long pendulous distal portion. The distinct shoot form resulting from bending is only somewhat similar to reports of other pendulous species, including weeping willow (*Salix babylonica*), weeping birch (*Betula pendula*), and weeping mulberry (*Morus alba* var. *pendula*) with newly developed shoots growing almost directly downwards (Reches et al. 1974), and twisted beech (*Fagus sylvatica* var. *tortuosa*) displaying a range in each plant's phenotypic expression of shoot bending, from nearly upright to completely pendulous (Thiébaut et al. 1985; 1992; 1998).

As crooked aspen shoots bent down, a degree of secondary bending was observed in many of the shoots in that the terminal portion of the shoot bent upwards with a relatively vertical orientation, demonstrating negative gravitropism. Similar development has been reported in the literature, with the most recently formed internodes at the tip of the shoot displaying a negative gravitropic response, while the oldest internodes show a positive gravitropic effect either due to active initial development downwards, as seen in trailing plants (Digby & Firn 1995), or biomechanical bending downwards from self-weight, as seen in lateral woody shoots (Fournier et al. 1994; Jirasek et al. 2000). Such secondary bending has been called gravitropic sign-reversal (Myers et al.

1994; Digby & Firn 1995), and indicates that the crooked aspen is responsive to the directional vector of gravity.

One of two general processes may be acting to cause the downward bending of shoots: *(i)* shoots bend passively under their own weight due to poor shoot strength (Zimmermann & Brown 1971; Niklas 1992; Edelin & Atger 1994; Kervella et al. 1994; Jirasek et al. 2000), as in Champagnat's model (Hallé et al. 1978), or *(ii)* shoot bending is an active process whereby the plant actively manipulates shoot orientation (Fisher & Stevenson 1981; Fournier et al. 1994; Hejnowicz 1997; Yamamoto et al. 2002; Alméras et al. 2004), somewhat similar to Mangenot's model (Hallé et al. 1978). In this study, both morphological and anatomical experiments were designed to test different aspects of both active and passive shoot bending hypotheses.

As crooked aspen shoots bent over the growing season many aspects of their general shoot morphology were similar to wild-type leader shoots. The shoots of both tree types grew in diameter proportional to their length in the process of adding lateral buds and leaves. It is well known that correlations between extension and radial growth are essential to maintain shoots with a particular orientation (Burk et al. 1983; Cannell et al. 1988). Moreover, because the basal internodes are the oldest, they initiate secondary growth first, resulting in a taper to the shoot (Kervella et al. 1994; Fournier et al. 1994). Shoot taper assists in supporting the shoots as the weight increases due to elongation. Wild-type and crooked aspen trees both developed tapered shoots; however, crooked aspen shoots became increasingly more tapered over the growing season

compared to wild-type shoots, that is, the crooked aspen had a greater rate of increase in basal diameter per unit shoot length. These findings were unlike reports in certain other pendulous species, including weeping Japanese cherry, that shoots are unable to support their weight because the thickening rate was less than the rate of elongation (Yoshida et al. 2000). It may be that increased diameter growth, and the resulting taper as observed in crooked aspen shoots, provide strength and rigidity (Burk et al. 1983; Cannell et al. 1988; Niklas 1992; Kervella et al. 1994). Such taper is often interpreted as a response to oblique orientation of developing branches to compensate for forces such as gravity and self-weight acting to cause passive shoot bending (Leiser & Kemper 1973; Fournier et al. 1994; Osler et al. 1996).

In the course of bending, the tissues of the basal portion of crooked aspen shoots that remained upright were the oldest, and were no longer actively extending. It appears that these tissues had enough secondary growth for support. However, despite greater taper across most of the length of crooked aspen shoots compared to wild-type shoots, bending occurred in those internodes distal to the upright basal portion. Initially these internodes were still actively extending, and therefore lateral differences in extension growth at this time could contribute to shoot bending, suggesting that an active process could be involved. With time, and shortly following the start of shoot bending, these internodes were fully extended. Therefore, at this stage of growth, shoot bending might be attributed to these internodes not being strong enough to support increased weight, resulting in further downward bending in a fashion consistent

with biomechanical passive bending of older internodes in leaning shoots (Jirasek et al. 2000). Overall, increased tissue production in crooked aspen shoots may not be sufficient to support the shoot above the level of the most basal internodes. Therefore, it may be possible that these added tissues are much weaker than structural tissues of the wild-type, and significantly more tissue is required to provide similar structural support.

An experiment designed to test whether crooked aspen shoots were as strong as those of the wild type revealed that wild-type and crooked aspen shoots bent similarly after weights were suspended from the shoot, based on the change in secant angle. However, the measured deflection from horizontal was greater in crooked aspen shoots after applying weight. Although these findings are inconclusive, they suggest the possibility that wild-type shoots were more rigid than crooked aspen shoots, even with less diameter growth. This latter observation contrasts with Cannell et al. (1988) who suggested that stems with less wood development (diameter growth) allow for greater deflection. In any event, to fully assess the potential for differences in shoot strength between tree types, further experiments would be necessary using different weights applied at several times throughout the growing season.

Another measure of shoot rigidity, or strength, is Young's modulus ($E$) (Cannell & Morgan 1987). An indirect measure of Young's modulus based on water content revealed that $E$ was significantly greater in wild-type aspen shoots compared to crooked aspen shoots, again indicating that crooked aspen shoots were less rigid than wild-type aspen shoots, and therefore less able to support

their weight. Both tree types had similar fresh weights at the time of sampling in this experiment; therefore, the weaker crooked aspen shoots would be expected to have a more oblique or bent form from passive bending, being less able to support the same weight as the wild type. Also, shoots with decreased *E*, are known to require increased diameter growth for self-support (Cannell et al. 1988), as was observed in the crooked aspen; however, shoots still bent, indicating again that either those added tissues were weak, or too minimal to prevent bending. It would be interesting to measure *E* at several times in the growing season to assess rigidity of the shoots of each tree type at different growth stages.

Progressive bending of crooked aspen shoots was also associated with several major differences in anatomy from the wild type. Anatomically, increases in shoot diameter were represented by an increase in vascular cambium activity, adding proportionally more secondary xylem tissue compared to secondary phloem (Chaffey 2002b; Helariutta & Bhalerao 2003). At approximately the mid-point of the growing season, close to the start of shoot bending, both wild-type and crooked aspen shoots began to add proportionally more structural tissues, in particular xylem fibres. Xylem fibres are known to provide structural support to the shoot with lignified cell walls (Okuyama et al. 1994; Dickison 2000; Plomion et al. 2001), helping to maintain a particular orientation. These structural tissues were added in a relatively symmetric manner in all shoot types until several weeks after the initiation of crooked aspen shoot bending. At this time, bent crooked aspen shoots were asymmetric, with significantly more xylem tissue on

the upper side compared to the lower side, and greater diameter growth overall than vertically oriented shoots. These findings are consistent with previous reports that vertically grown shoots are known to have a symmetric distribution of tissues (Raven et al. 1999; Chaffey 2002b), while shoots growing off vertical are known to develop with an asymmetrical arrangement of tissues (Wilson & Archer 1977; Fournier et al. 1994). Asymmetric development typically functions to maintain a particular orientation against forces such as gravity, that might act on the shoot (Edelin & Atger 1994; Alméras et al. 2004). Therefore, it appears that the vascular cambium is functioning normally in both wild-type and crooked aspen shoots, responding to their orientation in relation to gravity by adding cells asymmetrically (Wilson & Archer 1977; Fournier et al. 1994). Further evidence that asymmetry in the crooked aspen was a normal response to being oriented horizontally was revealed when vertically trained crooked aspen shoots did not have an asymmetric distribution of xylem. The mechanism involved in shoot bending is probably different from that responsible for increased cambial activity that leads to asymmetric growth.

Increased diameter growth is also known to function in active shoot bending by creating tissues with differential growth stresses that may act to reorient the shoot (Wilson & Archer 1977; Fournier et al. 1994; Yamamoto et al. 2002; Alméras et al. 2004). One such tissue is tension wood, characterized by the formation of gelatinous fibres (G-fibres) (Zobel & Van Buijtenen 1989; Jourez & Avella-Shaw 2003; Alméras et al. 2004). In this study, basic G-fibres were first found on the lower side but not on the upper side of crooked aspen shoots

shortly after the start of shoot bending. Crooked aspen shoots maintained those lateral differences in TW formation even after bending was complete. On the other hand, G-fibres were found throughout the secondary xylem tissue of all other shoot types (vertical and obliquely-angled wild-type aspen shoots, and vertically trained crooked aspen shoots). The gelatinous layer observed in these fibres was relatively young, as it has been reported to first form in the corner of cells (Raven et al. 1999). Typically it is reported that G-fibres of TW in older stems act to create tension within that tissue, thus bending the stem in the direction of that tension (Fisher & Stevenson 1981; Okuyama et al. 1994; Bamber 2001). TW is known to form throughout vertically oriented shoots as a mechanism to maintain vertical orientation (Wilson & Archer 1977; Mellerowicz et al. 2001; Jourez & Avella-Shaw 2003), and TW is commonly found in some obliquely oriented angiosperm stems. In some cases it is initially formed on the lower side to re-orient the stem in a more horizontal manner, and subsequently on the upper side to maintain a characteristic oblique orientation (Fisher & Stevenson 1981; Fournier et al. 1994; Jourez & Avella-Shaw 2003). Therefore, asymmetric differences in TW formation within bent crooked aspen shoots suggest two possible mechanisms for its the role in shoot bending: *(i)* TW formation on the lower side of shoots, with no TW on the upper side, is actively bending shoots downwards, and/or *(ii)* the lack of TW on the upper side of shoots is not compensating or reorienting the shoot, thus allowing for passive shoot bending. No lateral differences in TW formation were observed in vertically trained crooked aspen shoots, similar to the case for asymmetric growth,

suggesting that the distribution of TW within bent shoots was the result of their orientation, thus supporting the latter hypothesis. On the other hand, the fact that the timing of shoot bending coincided with the production of TW, and bent in the direction of asymmetric TW, provides circumstantial evidence of a possible active role in shoot bending during the growing season. However, an initially oblique orientation is required to signal the formation of these lateral differences. It has been hypothesized that TW may be non-functional during the growing season in young, current-year shoots (Hejnowicz 1967; 1997). In the crooked aspen all shoot bending occurred during the growing season, and little or no shoot bending was observed after the first growing season (Remphrey & Pearn 2003). Thus, its role in crooked aspen shoot bending remains unclear.

Asymmetric TW formation in oblique shoots of crooked aspen might have been caused by the lateral redistribution of hormonal signals involved in either promoting or inhibiting TW formation. The lateral distribution of auxin is known to influence tension wood development in that TW forms under low auxin concentrations (Wilson & Archer 1977), usually on the upper side of oblique angiosperm branches, relative to gravity. Such an interpretation does not agree with the observation that TW was only found on the lower side of the oblique crooked aspen shoots. Other studies have shown that experimentally applied auxins stopped the formation of TW on the upper side of leaning angiosperm shoots (Wilson & Archer 1977; Hellgren et al. 2004). Therefore, more TW on the lower side of crooked aspen shoots suggests that either auxin concentrations were high on the upper side of bent crooked aspen shoots or the tissues were

less responsive to auxin, thus inhibiting the formation of TW. Reches et al. (1974) found no unilateral accumulation of auxin and no TW on either side of pendulous weeping mulberry shoots; however, they reported an asymmetric distribution of both gibberelin and ABA, indicating that numerous hormonal mechanisms may be involved in the development of pendulous branches.

Along with adding more structural xylem tissues, existing primary phloem fibre cell walls were progressively lignified over the growing season, presumably to cope with increases in shoot length and self-weight (Den Outer 1993; Quilhó et al. 2000). There is circumstantial evidence that differences in phloem fibre anatomical patterns in crooked aspen shoots compared to wild-type shoots may have contributed to the bending behaviour. As was indicated earlier, sampled shoot internodes were still actively extending when bending first occurred. Phloem fibres throughout most of these sampled shoot types were only partly lignified with a central lumen; however, phloem fibres located on the lower side of bending crooked aspen shoots were fully lignified with no central lumen. This lignification could restrict extension growth (Mauseth 1988) on the lower side of the shoot at a relatively early stage of growth compared to the upper, while the upper side continued to extend, creating a pivot, and thus actively directing the shoot in an oblique direction, to ultimately bend downward. Reches et al. (1974) observed increased lignification on the lower side of pendulous weeping mulberry shoots, and reported that these tissues might be less responsive to increased amounts of auxin, thus decreasing extension growth on that side, causing shoots to bend.

After bending was complete all phloem fibres were fully lignified at the midpoint of wild-type shoots, but still only on the lower side of the major point of bend in crooked aspen shoots. Phloem fibres on the upper side were not fully lignified and maintained a central lumen. However, at this time these internodes were no longer actively extending, and therefore the observed lateral differences in phloem fibre anatomy at this stage were not actively involved in causing shoot bending. Instead, the differences in phloem fibre lignification at this stage may have resulted in reduced strength on the upper side, thus contributing to passive shoot bending. Phloem fibres have been reported to provide significant structural support in the form of long fibres that run the length of the shoot (Quilhó et al. 2000). These fibres contain the thickest, most lignified cell walls of all tissues in young aspen shoots (Den Outer 1993; Chaffey et al. 2000). Also, the bark, including phloem fibres, is known to play a major role in a shoot's ability to resist deflection, especially at a young age (Niklas 1999).

Lateral differences in phloem fibre lignification were also observed in vertically trained crooked aspen shoots. This suggests that these differences were characteristic of a postulated genetic mutation (Remphrey & Pearn 2003) in the crooked aspen and provides more evidence for a possible role in shoot bending. Investigation of longitudinal sections would be helpful in determining whether differences in cell length occurred on opposite sides of aspen shoots. Such information would help clarify whether extension growth was restricted on the lower side of crooked aspen shoots, or whether shoots were simply not strong enough and bent passively under their own weight.

A preliminary investigation for the possible role of apically produced auxin in controlling bending was conducted by removing the terminal leaves of developing crooked aspen shoots prior to shoot bending. These leaves are considered the most abundant source of terminally produced auxin (Leakey & Longman 1986). Less bending was observed in treated crooked aspen shoots, giving rise to the hypothesis that terminally produced hormones, particularly auxin, might play a signalling role in the development of the crooked form. As an indicator, it would be interesting to examine the anatomy of such shoots to see if TW formed on all sides of the shoot, as was found in wild-type and vertically trained crooked aspen shoots.

Near the end of the growing season crooked aspen shoot bending ceases and shoots maintain a relatively uniform orientation. Cessation of bending may be explained as the shoots finally forming sufficient support tissues to maintain a particular orientation without further bending (Edelin & Atger 1994; Kervella et al. 1994; Niklas 1999). Conversely, the shoots may have finally started to form tension wood on the upper side of bent shoots, holding them with a particular orientation (Fournier et al. 1994; Hejnowicz 1997; Alméras et al. 2004). Further anatomical analyses, employing techniques for sectioning older tissues, would be necessary. These hypotheses are consistent with the idea that the mechanisms for normal shoot primary and secondary development are present in crooked aspen shoots, but are altered in some way. Therefore bending occurs before the shoots are fully strengthened later in the growing season. Evidence for shoot strengthening, without the unilateral functioning of TW, can be observed in older

stems of crooked aspen crowns maintaining the same crooked orientation year after year (Remphrey & Pearn 2003).

It has been shown previously that bending of leading shoots in the crooked aspen sets in motion a series of developmental events leading to a crooked form (Remphrey & Pearn 2003). In this study it was also observed that the longest lateral shoots emerged from the highest point in relation to gravity along all shoots types; the midpoint along bent crooked aspen shoots and the shoot tip of wild-type and vertically suspended crooked aspen parent shoots. The pattern of shoot emergence confirms that daughter shoot vigour is dependent on shoot orientation in the crooked aspen, as has been reported for wild-type aspen shoots held off vertical (Jankiewicz et al. 1967; Jankiewicz & Stecki 1976), and that the response is truly gravimorphic in nature.

# 6.0 CONCLUSIONS

The results from this study effectively characterized the ontogeny of leading shoots of crooked aspen. The distinct shoot bending was described as a relatively rapid motion occurring in the internodes formed approximately midway through the growing season, with a subsequent and progressive downwards bending of all distal tissues. As the distal internodes became more and more pendulous, a degree of secondary bending upwards was observed in the terminal internodes of many shoots. These are the youngest internodes of developing crooked aspen shoots and they demonstrated a degree of negative gravitropism, indicating that the crooked aspen was responsive to the gravitational stimulus. The result was a crooked shoot, only somewhat similar to those of other pendulous species reported in the literature, with a somewhat upright basal portion, a shorter horizontal bending point, and a long pendulous distal portion, with a range of upward tropic bending at the shoot tip. Subsequent analysis of the processes involved in creating the bent form suggested two potential mechanisms: crooked aspen shoots may bend actively by differential elongation on either side of the shoot, or shoots may bend passively under their own weight, in a manner similar to reports of biomechanical bending of branches.

From the analysis of shoot morphology and form, the crooked aspen had larger diameters and greater taper at similar shoot lengths and therefore should be more rigid than the wild-type and resist bending. Also, anatomically the vascular cambium added proportionally more xylem fibres to improve strength,

and in an asymmetric manner relative to the direction of gravity. These findings are all consistent with reports of shoots developing with a lateral or oblique orientation, or as a result of an external bending stress, indicating that the mechanisms involved in each of these functions are properly reacting to shoot orientation. However, these mechanisms are apparently ineffective at preventing further bending, with the exception of the basal portion of crooked aspen shoots that maintained a relatively upright position. These internodes formed sufficient diameter growth for support, and because they have larger basal diameters than wild-type shoots, it indicates that significantly more tissues are required for support in shoots of the crooked aspen.

Early in development the active transport of water to the leaves created turgor pressure that was able to maintain the shoot in a relatively vertical manner. Also, there was little risk of passive bending at this stage as the shoots were still quite small and had not yet developed significant amounts of weight.

An analysis of shoot strength at the start of shoot bending was inconclusive but nevertheless it suggested that crooked aspen shoots might be slightly more flexible than the wild-type. The possibility of an active mechanism causing initial shoot bending is plausible as the internodes involved in shoot bending were still actively extending. Increased lignification of phloem fibres on the lower side may lead to restricted extension growth on the lower side of the shoot, creating a bending point. Moreover, the initial formation of tension wood on the lower side of crooked aspen shoots coincided with this initial bending, and was formed in a location that that could initiate active downwards bending.

Later in development, as the shoots increased in size and weight, the internodes at the major point of bend were no longer actively extending, and therefore lateral differences in phloem fibre lignification could not have resulted in further differential extension growth. Nevertheless, at this time, differences in phloem fibre cell wall lignification may have resulted in the inability of crooked aspen shoots to support their own weight, and bending may occur because of decreased strength and increased flexibility on the upper side of the shoot. These conclusions are consistent with results indicating that crooked aspen shoots were less rigid (more flexible) than wild-type shoots at the end of the growing season, thus allowing for passive bending.

Tension wood is usually reported as functioning after extension growth is complete and is considered to play a role in maintaining a certain orientation. The formation of rudimentary TW on the lower side of young bending shoots presents somewhat of an enigma. Lateral differences in TW formation within crooked aspen shoots would have either actively bent the shoots down, and/or conversely, the lack of TW on the upper side of bent shoots would not have prevented passive bending due to its inability to support the weight. The latter is a better explanation, as the active mechanism may be expected to continue to bend the shoots after the growing season, and in subsequent years. No such bending occurred; therefore the hypothesis of active bending caused by TW could be rejected unless a stronger opposing force acted to prevent subsequent shoot bending after the growing season.

Near the end of the growing season shoot bending stopped and an apparent solidification of tissues maintained the shoots with a particular orientation. A theory for the eventual fixing of crooked aspen shoot tissues is that the mechanisms involved in shoot strengthening are slow to form throughout the entire shoot, thus allowing for changes in form, including bending. Such has been reported for biomechanical models of older shoot tissues bending from self weight. One possible explanation for the fixing is that the crooked aspen finally formed adequate tissues to support the entire shoot, as was observed in the basal internodes earlier in the season. Moreover, existing shoot tissues, including phloem fibres, might have finally completely strengthened themselves, forming fully lignified cell walls on all sides of the shoot, providing equal support throughout; however, no such examinations were conducted in this study. In terms of TW formation, it is possible that G-fibres finally formed on the upper side of bent shoots, acting to maintain the crooked form, but a subsequent righting of oblique aspen stems would be expected and was not observed. Further examination of the phenomenon of end-of-season shoot fixing would be essential to determine how shoot bending is stopped, and would provide insight as to whether the mechanisms involved in shoot bending are the result of a complete or incomplete inhibition of regular developmental functions.

Whatever the mechanism responsible for the bent form of crooked aspen shoots, it is probably controlled by the lateral redistribution of certain hormones involved in extension growth, and/or cell wall development, in relation to gravity. Preliminary evidence existed that these hormones are transported basipetally, as

indirectly restricting terminal sources of hormones minimized shoot bending in the crooked aspen. Further physiological examinations of the lateral distribution of endogenous hormones within crooked aspen shoots would be valuable.

As an opening look at the behaviour of crooked aspen shoot development, progress was made in completely describing the act and timing of shoot bending. At first glance, morphologically, crooked aspen shoots appeared to react to their oblique orientation by forming tissues that should act to support the shoot. However, a novel anatomical investigation during shoot development revealed a distinctive distribution of several unique tissues that could be involved in the bending of shoots. An initially active process of shoot bending could have set in motion a progression of events that led to passive bending due to a delayed strengthening of internal tissues, the final result being the unique bent shoot form characteristic of the crooked aspen leading shoots.

## LITERATURE CITED

Alméras, T., Costes, E., and Salles, J.C. 2004. Identification of biomechanical factors involved in stem shape variability between apricot tree varieties. Ann. Bot. **93:** 455-468.

Alméras, T., Gril, J., and Costes, E. 2002. Bending of apricot tree branches under the weight of axillary growth: test of a mechanical model with experimental data. Trees **16:** 5-15.

Bamber, R.K. 2001. A general theory for the origin of growth stresses in reaction wood: how trees stay upright. IAWA J. **22:** 205-212.

Barlow, P.W. 1995. Gravity perception in plants: a multiplicity of systems derived by evolution? Plant Cell Environ. **18:** 951-962.

Borkowska, B. 1975. Distribution of stored $C^{14}$ assimilates in apple trees during their spring development. Fruit Sci. Rep. **2:** 1-11.

Borkowska, B. and Jankiewicz, L.S. 1972. The influence of gravity on bud development in apple trees and in poplars. Acta Agrobot. **25:** 185-194.

Bowes, B.G. 1996. A Color Atlas of Plant Structure. Iowa State University Press, Ames.

Boyd, J.D. 1977. Basic cause of differentiation of tension wood and compression wood. Aust. Forest Res. **7:** 121-143.

Brown, C.L., McAlpine, R.G., and Kormanik, P.P. 1967. Apical dominance and form in woody plants: a reappraisal. Am. J. Bot. **54:** 153-162.

Burk, T., Nelson, N.D., and Isebrands, J.G. 1983. Crown architecture of short-rotation, intensively cultured *Populus*. III. a model of first-order branch architecture. Can. J. For. Res. **13:** 1107-1116.

Cannell, M.G.R. and Morgan, J. 1987. Young's modulus of sections of living branches and tree trunks. Tree Physiol. **3:** 355-364.

Cannell, M.G.R., Morgan, J., and Murray, M.B. 1988. Diameters and dry weights of tree shoots: effects of young's modulus, taper, deflection and angle. Tree Physiol. **4:** 219-231.

Carlquist, S. 2001. Comparative Wood Anatomy: Systematic, Ecological, and Evolutionary Aspects of Dicotyledon Wood. Springer-Verlag, Berlin.

Ceulemans, R., Stettler, R.F., Hinckley, T.M., Isebrands, J.G., and Heilman, P.E. 1990. Crown architecture of *Populus* clones as determined by branch orientation and branch characteristics. Tree Physiol. **7:** 157-167.

Chaffey, N. 1999. Cambium: old challenges - new opportunities. Trees **13:** 138-151.

Chaffey, N. 2002a. Why is there so little research into the cell biology of the secondary vascular system of trees? New Phytol. **153:** 213-223.

Chaffey, N. 2002b. Wood Formation in Trees: Cell and Molecular Biology Techniques. Taylor & Francis, London.

Chaffey, N., Barlow, P., and Barnett, J. 2000. Structure-function relationships during secondary phloem development in an angiosperm tree, *Aesculus hippocastanum*: microtubules and cell walls. Tree Physiol. **20:** 777-786.

Champagnat, P. 1978. Formation of the trunk in woody plants. *In* Tropical Trees As Living Systems. *Edited by* P.B.Tomlinson and M.H.Zimmermann. Cambridge University Press, Cambridge. pp. 401-422.

Chen, R., Rosen, E., and Masson, P.H. 1999. Gravitropism in higher plants. Plant Physiol. **120:** 343-350.

Cline, M.G. 1991. Apical dominance. Bot. Rev. **57:** 318-358.

Correll, M.J. and Kiss, J.Z. 2001. Interactions between gravitropism and phototropism in plants. J. Plant Growth Regul. **20:** 226-243.

Davidson, C.G. and Remphrey, W.R. 1990. An analysis of architectural parameters of male and female *Fraxinus pennsylvanica* in relation to crown shape and crown location. Can. J. Bot. **68:** 2035-2043.

Den Outer, R.W. 1993. Evolutionary trends in secondary phloem anatomy of trees, shrubs and climbers from Africa (mainly Ivory Coast). Acta Bot. Neerl. **42:** 269-287.

Dickison, W.C. 2000. Integrative Plant Anatomy. Harcourt / Academic Press, San Diego.

Dickmann, D.I., Isebrands, J.G., Eckenwalder, J.E., and Richardson, J. 2001. Poplar Culture in North America. NRC Research Press, Ottawa.

Digby, J. and Firn, R.D. 1995. The gravitropic set-point angle (GSA): the identification of an important developmentally controlled variable governing plant architecture. Plant Cell Environ. **18:** 1434-1440.

Dirr, M.A. 1998. Manual of Woody Landscape Plants: Their Identification, Ornamental Characteristics, Culture, Propagation and Uses. Stipes Publishing, Champaign.

Donaldson, L.A. 2001. Lignification and lignin topochemistry - an ultrastructural view. Phytochem. **57:** 859.

Edelin, C. and Atger, C. 1994. Stem and root tree architecture: questions for plant biomechanics. Biomimetics **2:** 253-266.

Farrar, J.L. 1995. Trees in Canada. Fitzhenry & Whiteside Limited, Markham.

Feder, N. and O'Brien, T.P. 1968. Plant microtechnique: some principles and new methods. Am. J. Bot. **55:** 123-142.

Fisher, J.B. 1992. How predictive are computer simulations of tree architecture. Int. J. Plant Sci. **153:** S137-S146.

Fisher, J.B. and Stevenson, J.W. 1981. Occurrence of reaction wood in branches of dicotyledons and its role in tree architecture. Bot. Gaz. **142:** 82-95.

Fournier, M., Bailleres, H., and Chanson, B. 1994. Tree biomechanics: growth, cumulative prestresses, and reorientations. Biomimetics **2:** 229-251.

Fukaki, H., Wysocka-Diller, J., Kato, T., Fujisawa, H., Benfey, P.N., and Tasaka, M. 1998. Genetic evidence that the endodermis is essential for shoot gravitropism in *Arabidopsis thaliana*. Plant J. **14:** 425-430.

Grunwald, C., Ruel, K., Joseleau, J.P., and Fladung, M. 2001. Morphology, wood structure and cell wall composition of *rolC* transgenic and non-transformed aspen trees. Trees **15:** 503-517.

Guedes, M. 1982. A simpler morphological system of tree and shrub architecture. Phytomorphology **32:** 1-14.

Hallé, F., Oldeman, R.A.A., and Tomlinson, P.B. 1978. Tropical Trees and Forests: an Architectural Analysis. Springer-Verlag, Berlin.

Hangarter, R.P. 1997. Gravity, light and plant form. Plant Cell Environ. **20:** 796-800.

Hejnowicz, Z. 1967. Some observations on the mechanism of orientation movements in woody stems. Am. J. Bot. **54:** 684-689.

Hejnowicz, Z. 1997. Graviresponses in herbs and trees: a major role for the redistribution of tissue and growth stresses. Planta **203:** S136-S146.

Helariutta, Y. and Bhalerao, R. 2003. Between xylem and phloem: the genetic control of cambial activity in plants. Plant biol. **5:** 465-472.

Hellgren, J.M., Olofsson, K., and Sundberg, B. 2004. Patterns of auxin distribution during gravitational induction of reaction wood in poplar and pine. Plant Physiol. **135:** 212.

Isebrands, J.G. and Nelson, N.D. 1982. Crown architecture of short-rotation, intensively cultured *Populus.* II. branch morphology and distribution of leaves within the crown of *Populus* 'Tristis' as related to biomass production. Can. J. For. Res. **12:** 853-864.

Isnard, S., Rowe, N., and Speck, T. 2003. Growth habit and mechanical architecture of the sand dune-adapted climber *Clematis flammula* var. *maritima* L. Ann. Bot. **91:** 407-417.

Jankiewicz, L.S., Srzednicka, W., and Borkowska, B. 1967. Gravimorphism in the poplar (*Populus* sp.). B. Acad. Pol. Sci. Biol. **15:** 111-113.

Jankiewicz, L.S. and Stecki, Z.J. 1976. Some mechanisms responsible for differences in tree form. *In* Tree Physiology and Yield Improvement. *Edited by* M.G.R.Cannell and F.T.Last. Academic Press, London. pp. 157-172.

Jirasek, C., Prusinkiewicz, P. W., and Moulia, B.2000. Integrating biomechanics into developmental plant models expressed using L-systems. *In* Plant Biomechanics 2000, *Edited by* H.C.Spatz and T.Speck. Georg Thieme Verlag, Stuttgart. pp. 615-624.

Jourez, B. and Avella-Shaw, T. 2003. Effet de la durée d'application d'un stimulus gravitationnel sur la formation de bois de tension et de bois opposé dans de jeunes pousses de peuplier (*Populus euramericana* cv 'Ghoy'). Ann. For. Sci. **60:** 31-41.

Jourez, B., Riboux, A., and Leclercq, A. 2001. Anatomical characteristics of tension wood and opposite wood in young inclined stems of poplar (*Populus euramericana* cv 'Ghoy'). IAWA J. **22:** 133-157.

Jourez, B., Vaianopoulos, C., and Hebert, J. 2003. Effet de l'intensité d'un stimulus gravitationnel induit artificiellement sur la croissance et la formation du bois de tension dans de jeunes pousses de peuplier (*Populus euramericana* cv 'Ghoy'). Ann. For. Sci. **60:** 789-802.

Junghans, U., Langenfeld-Heyser, R., Polle, A., and Teichmann, T. 2004. Effect of auxin transport inhibitors and ethylene on the wood anatomy of poplar. Plant biol. **6:** 22-29.

Karwowski, R. and Prusinkiewicz, P. W.2004. The L-system-based plant-modeling environment L-studio 4.0. *In* Proceedings of the 4th International Workshop on Functional-Structural Plant Models, Montpellier, France. pp. 403-405.

Kervella, J., Pages, L., and Genard, M. 1994. Genotypic differences in the length-diameter relationship of branches of one-year-old peach and nectarine trees. J. Amer. Soc. Hort. Sci. **119:** 616-619.

Ko, J.H., Han, K.H., Park, S., and Yang, J. 2004. Plant body weight-induced secondary growth in *Arabidopsis* and its transcription phenotype revealed by whole-transcriptome profiling. Plant Physiol. **135:** 1069-1083.

Krishnamurthy, K.V., Venugopal, N., Nandagopalan, V., Hariharan, Y., and Sivakumari, A. 1997. Tension phloem in some legumes. J. Plant Anat. Morph. **7:** 20-23.

Larson, P.R. 1965. Stem form of young *Larix* as influenced by wind and pruning. For. Sci. **11:** 412-424.

Leakey, R.R.B. and Longman, K.A. 1986. Physiological, environmental and genetic variation in apical dominance as determined by decapitation in *Triplochiton scleroxylon*. Tree Physiol. **1:** 193-207.

Leiser, A.T. and Kemper, J.D. 1973. Analysis of stress distribution in the sapling tree trunk. J. Amer. Soc. Hort. Sci. **98:** 164-170.

Lindenmayer, A. 1968a. Mathematical models for cellular interactions in development. I. filaments and one-sided inputs. J. Theor. Biol. **18:** 280-299.

Lindenmayer, A. 1968b. Mathematical models for cellular interactions in development. II. simple and branching filaments with two-sided inputs. J. Theor. Biol. **18:** 300-315.

Little, C.H.A. and Lavigne, M.B. 2002. Gravimorphism in current-year shoots of *Abies balsamea*: involvement of compensatory growth, indole-3-acetic acid transport and compression wood formation. Tree Physiol. **22:** 311-320.

Maini, J.S. 1966. Apical growth of *Populus* spp. II. relative growth potential of apical and lateral buds. Can. J. Bot. **44:** 1581-1590.

Mauseth, J.D. 1988. Plant Anatomy. The Benjamin/Cummings Publishing Company, Inc., Menlo Park, California.

Mellerowicz, E.J., Baucher, M., Sundberg, B., and Boerjan, W. 2001. Unravelling cell wall formation in the woody dicot stem. Plant Mol. Biol. **47:** 239-274.

Mia, A.J. 1968. Organization of tension wood fibres with special reference to the gelatinous layer in *Populus tremuloides* Michx. Wood Sci. Technol. **1:** 105-115.

Millet, J., Bouchard, A., and Edelin, C. 1999. Relationship between architecture and successional status of trees in the temperate deciduous forest. Ecoscience **6:** 187-203.

Myers, A.B., Firn, R.D., and Digby, J. 1994. Gravitropic sign reversal - a fundamental feature of the gravitropic perception or response mechanisms in some plant organs. J. Exp. Bot. **45:** 77-83.

Nakamura, T., Saotome, M., and Tanaka, H. 1995. Weeping habit and gibberellin in *Prunus*. Acta Hort. **394:** 273-280.

Niklas, K.J. 1992. Plant biomechanics: an engineering approach to plant form and function. *In* Plant Biomechanics. Chicago Press, Chicago. pp. 420-423.

Niklas, K.J. 1999. The mechanical role of bark. Am. J. Bot. **86:** 465-469.

O'Brien, T.P. and McCully, M.E. 1981. The Study of Plant Structure Principles and Selected Methods. Termarcarphi Pty Ltd., Melbourne.

Okuyama, T., Yamamoto, H., and Yoshida, M. 1994. Growth stresses in tension wood: role of microfibrils and lignification. Ann. Sci. For. **51:** 291-300.

Osler, G.H.R., West, P.W., and Downes, G.M. 1996. Effects of bending stress on taper and growth of stems of young *Eucalyptus regnans* trees. Trees **10:** 239-246.

Perala, D.A. 1990. *Populus tremuloides* Michx. quaking aspen. *In* Silvics of North America. *Edited by* R.M.Burns and B.H.Honkala. Forest Service, United States Department of Agriculture, Washington. pp. 555-567.

Plomion, C., Leprovost, G., and Stokes, A. 2001. Wood formation in trees. Plant Physiol. **127:** 1513-1523.

Prusinkiewicz, P.W. and Lindenmayer, A. 1990. The Algorithmic Beauty of Plants. Springer-Verlag, New York.

Prusinkiewicz, P.W., Remphrey, W.R., Davidson, C.G., and Hammel, M.S. 1994. Modeling the architecture of expanding *Fraxinus pennsylvanica* shoots using L-systems. Can. J. Bot. **72:** 701-714.

Quilhó, T., Pereira, H., and Richter, H.G. 2000. Within-tree variation in phloem cell dimensions and proportions in *Eucalyptus globulus*. IAWA J. **21:** 31-40.

Ranjeva, R., Graziana, A., and Mazars, C. 1999. Plant graviperception and gravitropism: a newcomer's view. FASEB J. **13:** S135-S141.

Raven, P.H., Evert, R.F., and Eichhord, S.E. 1999. Biology of Plants. W.H. Freeman and Company, New York.

Reches, S., Leshem, Y., and Wurzburger, J. 1974. On hormones and weeping: asymmetric hormone distribution and the pendulous growth habit of the weeping mulberry, *Morus alba* var. *Pendula*. New Phytol. **73:** 841-846.

Remphrey, W.R., Bartlett, G.A., and Davidson, C.G. 2002. Shoot morphology and fate of buds in relation to crown location in young *Fraxinus pennsylvanica* var. *subintegerrima*. Can. J. Bot. **80:** 1274-1282.

Remphrey, W.R. and Pearn, L.P. 2003. Crown development of a clone of *Populus tremuloides* exhibiting "crooked" architecture and a comparison with wild-type trees. Can. J. Bot. **81:** 345-359.

Remphrey, W.R. and Powell, G.R. 1984. Crown architecture of *Larix laricina* saplings: quantitative analysis and modelling of (nonsylleptic) order 1 branching in relation to development of the main stem. Can. J. Bot. **62:** 1904-1915.

Ruzin, S.E. 1999. Plant Microtechnique and Microscopy. Oxford University Press, New York.

Sack, F.D. 1991. Plant gravity sensing. Int. Rev. Cytol. **127:** 193-252.

Salisbury, F.B. and Ross, C.W. 1992. Plant physiology. Wadsworth publishing company, Belmont, California.

Sievers, A., Buchen, B., and Hodlick, D. 1996. Gravity sensing in tip-growing cells. Trends Plant Sci. **1:** 273-279.

Simpson, W.T. Specific gravity, moisture content, and density relationship for wood. FPL-GTR-76, 1-13. 1993. Madison, Wisconsin, U.S. Department of Agriculture, Forest Service, Forest Products Laboratory.

Smirnova, O.V., Chistyakova, A.A., Zaugolnova, L.B., Evstigneev, O.I., Popadiouk, R.V., and Romanovsky, A.M. 1999. Ontogeny of a tree. Botanicheskii-Zhurnal **84:** 8-20.

Speck, T. 1994. Bending stability of plant stems: ontogenetical, ecological, and phylogenetical aspects. Biomimetics **2:** 109-128.

Srebotnik, E. and Messner, K. 1994. A simple method that uses differential staining and light microscopy to assess the selectivity of wood delignification by white rot fungi. Appl. Environ. Microb. **60:** 1383-1386.

Stasolla, C., Loukanina, N., Ashihara, H., Yeung, E.C., and Thorpe, T.A. 2002. Pyrimidine nucleotide and nucleic acid synthesis in embryos and megagametophytes of white spruce (*Picea glauca*) during germination. Physiol. Plantarum **115:** 155-165.

Takahashi, K., Seino, T., and Kohyama, T. 2001. Responses to canopy openings in architectural development of saplings in eight deciduous broad-leaved tree species. Can. J. For. Res. **31:** 1336-1347.

Thiébaut, B., Bujon, P., Haddad, S., Comps, B., and Mercier, J. 1992. Morphologie et architecture des hêtres tortillards à Verzy (Fagus sylvatica var. tortuosa). Can. J. Bot. **71:** 848-862.

Thiébaut, B., Cuguen, J., and Dupré, S. 1985. Architecture des jeunes hêtres *Fagus sylvatica* L. Can. J. Bot. **63:** 2100-2110.

Thiébaut, B., Garniaux, O., Comps, B., and Muller, C. 1998. Descendances maternelles de hêtre tortillard (*Fagus sylvatica* L. var. *tortuosa* Pépin); développement au cours des quatre premières années: I. fertilité, vigueur et apparition du phénotype tortillard. Can. J. Bot. **76:** 12-22.

Vazquez-Cooz, I. and Meyer, R.W. 2002. A differential staining method to identify lignified and unlignified tissues. Biotech. Histochem. **77:** 277-282.

Wareing, P.F. and Nasr, T. 1961. Gravimorphism in trees. 1. effects of gravity on growth and apical dominance in fruit trees. Ann. Bot. **25:** 321-340.

Wilson, B.F. 1997. Response to stem bending in forest shrubs: stem or shoot reorientation and shoot release. Can. J. Bot. **75:** 1643-1648.

Wilson, B.F. 1998. Branches versus stems in woody plants: control of branch diameter growth and angle. Can. J. Bot. **76:** 1852-1856.

Wilson, B.F. 2000. Apical control of branch growth and angle in woody plants. Am. J. Bot. **87:** 601-607.

Wilson, B.F. and Archer, R.R. 1977. Reaction wood: induction and mechanical action. Ann. Rev. Plant Physio. **28:** 23-43.

Wilson, B.F. and Archer, R.R. 1983. Apical control of branch movements and tension wood in black cherry and white ash trees. Can. J. For. Res. **13:** 594-600.

Wilson, B.F. and Gartner, B.L. 2002. Effects of phloem girdling in conifers on apical control of branches, growth allocation and air in wood. Tree Physiol. **22:** 347-353.

Yamamoto, H., Yoshida, M., and Okuyama, T. 2002. Growth stress controls negative gravitropism in woody plant stems. Planta **216:** 280-292.

Yeung, E.C. 1999. The use of histology in the study of plant tissue culture systems - some practical comments. In Vitro Cell. Dev. Biol. Plant **35:** 137-143.

Yoshida, M., Yamamoto, H., and Okuyama, T. 2000. Estimating the equilibrium position by measuring growth stress in weeping branches of *Prunus spachiana* Kitamura f. *spachiana* cv. *Plenarosea*. J. Wood Sci. **46:** 59-62.

Zimmermann, M.H. and Brown, C.L. 1971. Trees Structure and Function. Springer-Verlag, New York.

Zipse, A., Mattheck, C., Grabe, D., and Gardiner, B. 1998. The effect of wind on the mechanical properties of the wood of beech (*Fagus sylvatica* L.) growing in the borders of Scotland. Arboric. J. **22:** 247-257.

Zobel, B.J. and Van Buijtenen, J.P. 1989. Wood Variation: Its Causes and Control. Springer-Verlag, Berlin.