How Anatomical and Morphological Characteristics Affect the Flexural Properties of two Angiosperm Species at the Sapling Stage

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The sapling stage is an important phase due to maintaining plant growth, stability, and survival over the life cycle of trees. However, there are limited investigations in the literature related to both growth and stability of different tree species. This study thus investigated how different tree species at the sapling stage showed different anatomical, morphological, and flexural traits despite being of similar age and growing under the same environmental conditions. The variation of sapling properties was determined in two deciduous tree species: common oak (*Quercus robur* L.) and Oriental beech (*Fagus orientalis* Lipsky). The results of anatomical and morphological measurements showed that the highest average values of ray length, ray width, pith radius, pith%, bark%, and node numbers were obtained in oak saplings, whereas average ring width, number of rays, and wood% were found to be higher in beech saplings. Oak also exhibited better functional stability in its saplings. The flexural properties were almost 60% greater in oak stems than beech stems. The variations in flexural properties were explained by the morphological and anatomical traits since stability was positively correlated with pith radius, pith%, and bark% and negatively correlated with the number of rays and wood%.

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INTRODUCTION

The development, growth, and survival of trees can display great variation in species to species and in individual trees of the same species. This is the case even with trees of similar age and trees of a species growing under the same habitat. The variations in trees closely interact with environmental and climatic factors that develop species-based ecology (Beeckman 2016). Trees are exposed to many physiological (*i.e.*, drought stress, frost), biological (*i.e.* fungal infection), and mechanical (wind exposure, pull of gravity, and self-weight) stresses in their longer life span (Badel *et al.* 2015). However, many tree species could remain standing and survive even in unfavorable habitats and conditions over the years due to their favorable adaptative and survival strategies (Niklas and Spatz 2010). In harsh and extreme environments, trees modify their anatomical and morphological structures to determine how trees grow, develop, and remain strong in the existing environment. Thus, the adaptation in morphology and anatomy is a local phenomenon for each tree species and determines tree performance in its local environment (Dai 2019).

Trees in their juvenile (early) life stage of the growth, known as a sapling, are highly susceptible to environmental and climatic conditions because the growth of young saplings depends on both physiological and biomechanical changes (Harper 1977; Kitajima and

Fenner 2000; Wang *et al.* 2017). During the growth period, saplings are exposed to different loads that mostly originate from gravity, wind, snow, and rainfall, so they have the greatest potential to die. However, the mortality and survival of saplings depends on their ability to adapt to selective stresses over time. The morphology of tree species, therefore, has considerable adaptation or improvement response for the natural selection of species, successful plant growth, and tree stability (Anten *et al.* 2009). Due to environmental conditions and ecological pressures, trees could adapt their morphological structure by altering the shape, form, size, and dimension of their organs between and within species and individuals. Wind is one of the largest loads on the stem and branches, and it can greatly affect the tree performance (Ennos 1997; Vollsinger *et al.* 2005; Eugster 2008; Utsumi *et al.* 2010; Telewski 2012). The most common functional trait in tree morphology is changing size and dimension to overcome all stresses. Trees grow both in height (increasing height known as primary growth) and diameter (increasing in thickness known as secondary growth) (Haberlandt 1928; Thomas 2000). As the tree grows, the height and diameter differ allometrically in the organs of trees to keep tree architecture safe, such that when the tree grows in height, the diameter decreases from the base (near root) to the tips (apex). The difference in the size of length and diameter is known as tapering, which is an allometric property of the tree (Eloy *et al.* 2017). Tapering enables hydraulic safety, flexibility, and mechanical support against wind-induced bending stresses, and thus it can be regarded as a long-term adaptation strategy of trees to their local environmental conditions (Putz *et al.* 1983; Ennos 2001; Hacke *et al.* 2001; Sterck *et al.* 2005; Eloy *et al.* 2017; Özden *et al*. 2017).

The stem is the main organ of the tree, and it provides mainly mechanical support, conducting water and minerals up from the roots. The structure of stems is generally characterized by distinctive nodes and internodes. The internode is the stem region and interval between two nodes. Particularly, internode length is an important morphological trait, since it is mainly responsible for overall tree performance and height growth, which are affected by the local environments. However, the length of the internode varies considerably between tree species and within individuals. The internode length, therefore, displays how such trees adjust or respond to environmental and climatic factors (Poorter 2001). The trees grown under high levels of light have shorter shoots and internodes, but producing more branches in comparison to trees grown at low levels of light (Fisher and Honda 1979a,b; Lambers and Chapin 1998). Biomechanically, wind-induced stresses change the morphological structure of trees considerably at the sapling stage, since stem and internode extensions are decreased by strong winds. Trees therefore could likely grow more radially rather than in height to resist wind-induced bending stress, and this makes the stem structure flexible (Ennos 1993; Nagashima and Hikosaka 2011). Saplings are generally described as the smaller trees that are provided with an evolutionary advantage in stems relative to avoiding brittle failure against wind-induced bending stresses. This is because taller trees have a greater number of branches and larger canopies, which are exposed to higher wind-speeds at all levels; thus, taller trees face greater failures than smaller or younger ones (Niklas 1994; Niklas and Spatz 2000; Bruchert and Gardiner 2006). Morphologically, saplings are also thinner in diameter than taller trees, which makes saplings more flexible, but less solid. Due to its flexibility, the stem of a sapling can bend away from the wind to resist the influence of wind loading and to minimize the risk of stem failure or falling. In young trees or saplings, stiffness is also important, because the height and straightness of the stem are the outcomes of mainly environmental influences (Niklas 1992; Warren *et al.* 2009; Onoda *et al.* 2010; Weber *et al.* 2017). The stiffness of the tree is thus an ecological parameter that shows the mechanical response of the tree to environmental loadings.

Nodes are also an important structural component in stems, because buds, leaves, and branching twigs originate from the nodes. Therefore, they act as "constriction zones" in the stem, providing less cavitation (Zimmermann 1978a,b; Zimmermann and Sperry 1983). Furthermore, nodes provide healing, structural support, biological process, and hydraulic conductivity in the stem during the growing season (Zimmermann and Sperry 1983; Salleo and Lo Gullo 1986; Lo Gullo *et al.* 1995; Tyree and Zimmermann 2002). Previous studies showed that nodes are significant morphological traits of trees to provide mechanical support (Bergman *et al.* 2009; Caringella *et al.* 2014; Özden and Ennos 2018). A study by Özden and Ennos (2018) recently found that the mechanical properties of coppice and branch stems were negatively affected by a higher frequency of leaf nodes. In their study, sycamore and ash had more leaf nodes in their branches than coppices, so the flexural modulus was lower in their branches. The local environment and site conditions also affect the wood anatomical traits. In wood anatomy, the essential structure in a stem is composed of a woody skeleton.

Wood is known botanically as the xylem tissue that forms the bulk of the stem of a woody plant and is produced by the vascular cambium. The wood is made up of different elongated cells, which are tracheids, fibres, vessels, parenchyma cells, and ray cells. Each cell type has a specified task in plant establishment, survival, and stability. Tracheids are responsible for water transportation and mechanical support in gymnosperms; vessels provide water conduction in angiosperms; fibres are responsible for the mechanical support in angiosperms; and ray cells store sugar to help photosynthesis (Thomas 2000; Bowyer *et al.* 2003). Trees may adapt their nature by modifying the shape and arrangement of cells, size and dimension of cells, and the amount of chemical components in the cell wall layers (Gibson 2012; Gardiner *et al.* 2016). The annual growth ring is also an important anatomical trait of a tree, which indicates the amount of growth in one year. Tree annual growth rings have occurred each year after the conclusion of primary wood tissue production. Each ring comprises earlywood (EW) and latewood (LW) zones. The EW forms early in the spring, and the cells have a large diameter and thin-walled wood, so the wood is less dense. The LW forms at the end of the growing season and cells have a smaller diameter with thicker cell walls; thus the wood is denser (Gibson *et al.* 1988; Dresch and Dinwoodie 1996; Thomas 2000; Barnett and Jeronimidis 2003). The width of annual growth shows the response of the tree to growing conditions, such that wider annual rings show tree grown in better conditions and narrower annual rings display tree grown in worse growing conditions.

Tree ring features are linked to the wood density, since density is the outcome of the processes in the volume growth of wood (*i.e.* cell division and enlargement, EW and LW proportions) and biomass accumulation processes (cell wall thickening) during the growth season (MacDonald and Hubert 2002; Thomas *et al.* 2007). Several studies have reported that wood density is a determinative property for the physical and mechanical properties of wood. Previous studies suggested that wood density and mechanical properties were positively correlated with each other; that is, the increase of density makes the wood stronger and stiffer (Ifju and Kennedy 1962; Petterson and Bodig 1983; Ashby *et al.* 1985; Smith and Chui 1994; Özden and Ennos 2018). However, in young trees, growth rate and wood density were found to be negatively correlated (Enquist *et al.* 1999; Roderick 2000; Muller-Landau 2004; King *et al.* 2005), such that fast-growing trees would produce less dense wood than slow-growing species. Changing morpho-anatomical characteristics, therefore, allowing saplings or trees to efficiently resist wind-induced loadings.

To date, many studies have only examined morphological or anatomical traits of the trunk, branch, and coppice stem in trees (Spatz et al. 1990, 1993, 1995; Schulgasser and Witztum 1992; Niklas 1997; Warren *et al.* 2009; Genet *et al.* 2013; Beeckman 2016; Wang *et al.* 2017; Özden and Ennos 2018). However, there has been less detailed study on the growth variation and stability in the sapling of different tree species in the same growing environment. Different tree species in the sapling stage may show different survival strategies to cope with environmental stresses because each species has different morphology and anatomy. The primary objectives of this study are therefore to examine the following: (1) to evaluate whether different tree species growing under the same habitat and in similar age differ in their morphological, anatomical, and mechanical traits; and (2) to determine how morphological and anatomical characteristics affect the flexural properties of saplings.

EXPERIMENTAL

Materials

Study site and material collection

The species common oak (*Quercus robur* L.) and Oriental beech (*Fagus orientalis* Lipsky) were chosen for the following reasons: (1) similar age and distributed in the same geographical area; (2) have similar ecological niches; (3) produced by natural regeneration; (4) had straight stems and upright growth; (5) no competition between individuals; (6) and have economic and environmental importance. These are the most common tree species in Turkish forests. Oak is a moderately shade-tolerant species. However, the oak tree is shade tolerant when it is young, but it becomes intolerant with increasing age. Oak trees grow better on fertile and moist soils and develop a deep root system (taproot) that allows rapid shoot growth (Vranckx *et al.* 2014; Eaton *et al.* 2016). Oak also shows great competitive ability in its seedling and sapling. Beech is the most shade-tolerant species and grows in mixtures with other tree species. It is a successful competitor with other species even in the closed canopy, and thus its growth rate and regeneration are considerably greater than other trees. The growth of the beech tree is well developed on deep and moist soils (Carpenter 1974; Szwagrzyk *et al.* 2012; Haghshenas *et al.* 2016). Oak and beech saplings, therefore, require quite a similar environment for growing, and both saplings were dominant tree species in the planted site. The saplings were harvested in winter (December 2017) because winter is a dormant season, and the formation of EW and LW portions was induced. The study site was located at İğdir, Kastamonu, Turkey (N 41°19′, E 33°11′), with an altitude of 1350 m (a.s.l). The species were obtained at a similar age (5 years old) to keep all the factors the same. The site is located within the Euro-Siberian phytogeographical region and characterized by a terrestrial Black Sea climate with warm, wet summers and cool to cold, wet winters. The annual precipitation was 465.4 mm, and the average temperature was 11 ºC. Each sapling was cut in a fresh state, placed in a plastic bag to reduce sap loss, and stored at 4 °C to retain their freshness before dissection and testing.

Methods

Morphological characteristics

The plant material was collected during December 2017 from 40 randomly selected individual oak and beech saplings. The mean height of stems was approximately 70 to 80 cm, and the diameter at the central point of the stem's length averaged 9.6 mm. Before the experiments, for each section the following measurements were made: the stem height (in cm), the stem diameter (in mm) over the bark at the mid-point of the length, bark radial diameter (in mm), wood radial diameter (in mm), pith diameter (in mm), and node numbers. Measurements were made to the nearest 0.1 mm with a digital caliper. The stem height was measured from the shortest distance of the above-ground level, expressed in meters. The stem diameter was obtained in the horizontal and vertical plane of the stems, then the average diameter was taken. The area of each stem composed of the pith (pith %), bark (bark%), and wood (wood%) were calculated using these measurements. Node numbers were counted through the length of stems then recorded.

Anatomical and physical characteristics

The saplings of two different hardwood tree species were tested. The species have a different arrangement of vessel elements: ring-porous common oak (*Quercus robur* L.) and diffuse-porous oriental beech (*Fagus orientalis* Lipsky). The rays of both oak and beech were multiseriate. The widths of each annual ring were measured (in mm), and the average ring width was taken. The measurements were taken from the stem circumference of saplings towards the stem pith.

For anatomical cell characterization, the stems of seedlings were cut into small wood sticks (approximately 1 cm in height). The small wood samples were boiled in water then were exposed to distilled water, glycerol, and ethanol. Softened specimens were cut into thin slices using a sliding microtome. The specimens were sectioned in transverse and tangential sections to measure annual ring width, ray numbers per mm^2, and ray height/width. The sections were stained with safranin for microscopic analysis. A total of 25 measurements were performed for each cell anatomical characterization. The cells were analyzed using Leica DM750 light microscope (Heerbrugg, Switzerland) equipped with Leica Application Suite Computer Image Analysis Software, which allowed clear photographs. Ray cell sizes and numbers were measured and counted using a LAS EZ Image Analysis Software count tool.

Wood density was determined using the water displacement method (Archimedes' principle). Wood samples were soaked into the water to ensure all were fully hydrated, then each piece of wood was immersed in a beaker of water placed on top of an electronic balance. To determine the green volume of a piece of wood, the increase in weight was divided by the density of water. The samples were kept in an oven at 103 ºC until dry then weighed. Finally, oven-dry weight (0% moisture content) of the sample was divided by the saturated volume to obtain wood density (Hacke *et al.* 2000; Barnett and Jeronimidis 2003).

Three-point bending measurements

During winter 2017, each sapling was harvested and then stored in a cold room at 4 °C to keep stem segments in green condition until mechanical tests. The stems were 70 to 80 cm in length, and their tips were thinner and fragile. A total 40 stems were cut into 30 cm segments from base to tip point (thinner tips were mostly cut). The stem segments were used to calculate bending strength (M_{max}), flexural or Young's modulus (E), flexural stiffness (EI), maximum longitudinal bending stress (σ_{Bmax}), and specific stiffness

(E/density). Each stem (including bark, phloem, xylem, and pith) was subjected to three-point flexural tests using a Shimadzu autograph AGS-X universal testing machine (Kyoto, Japan) equipped with a 1 kN load cell and attached to an interfacing computer. The three-point bending test was conducted to observe the flexural behavior of species due to its many advantages. These are simplicity while performing, allowing a physical observation of the material's response to stress, and providing substantial information regarding the deformation behavior of the process.

Young's modulus or modulus of elasticity (E) is one of the important mechanical properties of wood that can be characterized as the wood stiffness and is a size-independent parameter. Stiffness is the elastic property of wood that simply describes the resistance of wood to deformation within the linear range and is the initial slope of the stress-strain curve. E can be calculated using the following formula (Burgert 2006; Dahle and Grabosky 2010; Ennos 2012),

$$E = \frac{d\sigma}{d\varepsilon} \tag{1}$$

where dσ is the stress (which is the load per unit area) and d$\mathcal{E}$ is the strain (which is the elongation under force per unit length).

The shear effect complicates the flexural stiffness calculations from the three-point bending tests. To minimize this effect, therefore, a minimum span-to-depth ratio of 15:20 was used (Vincent 1992), so the shear effect can be neglected. Span length was chosen in the required span-to-depth ratio. The crosshead was lowered to a speed of 35 mm min^{-1}, while the required force was simultaneously measured using a 1 kN load cell. The maximum loads (F_{max}) were obtained by the three-point bending tests. Flexural rigidity EI is a structural property and represents the composite measure of overall bending stiffness. It is calculated in units of N m^2 using Eq. 2,

$$EI = \frac{dF}{dx}\frac{L^3}{48} \tag{2}$$

where dF/dx is the initial slope of the load-displacement curve of the beam and L is the span length of the specimen between the lower supports. The dF/dx value was estimated using Excel. The force and displacement were first plotted, and the slope of the initial linear region of the curve was taken. Bending strength is also a structural property of the beam. Mathematically, bending strength M_{max} (N.m) is calculated using the following equation,

$$M_{max} = F_{max}\, L/4 \tag{3}$$

where F_{max} is the maximum force.

The sapling stems were nearly circular in their cross-sections at the internodes, while the nodes were more likely to have elliptical cross-sections. At the internodes, the ratios in diameter between the parallel and perpendicular planes were 1.04%. The cross-sections of stems were thus considered to be almost circular, particularly at the internodes. Flexural rigidity EI is directly affected by the second-moment area of the beam (I). I is a measure of the efficiency of cross-sections when bent in the vertical plane (Ennos 2012). The second moment of area of a circular beam is calculated using the equation, where R is the radius of the outer stem and r is the radius of the pith, as shown in Eq. 4.

$$I = \frac{\pi\,(R^4 - r^4)}{4} \tag{4}$$

Finally, maximum longitudinal bending stress (σ_{Bmax}) of the material in bending (with units N m^{-2}) is given by Eq. 5,

$$\sigma_{Bmax} = \frac{M\max X\ r}{I} \quad (5)$$

Specific stiffness or specific E was also calculated to compare wood stiffness (E) to density ratio (Nocetti *et al.* 2010; Weber *et al.* 2017), as shown in Eq. 6.

$$Specific\ stiffness = \frac{E}{density} \quad (6)$$

Statistical analysis

To determine the effect of tree species on morphological (stem diameter, node numbers, pith area, bark area, and wood area), anatomical (average annual ring width, ray numbers, ray length and ray width), wood density, and flexural properties (M_{max}, E, EI, *max. long. stress*, *specific stiffness*), statistical analysis was performed using the SPSS 19.0 statistical software package. One-way analysis of variance (ANOVA) was used to test the statistical significance in different properties between two species at the $\alpha = 0.05$ level. The linear regression tests were also conducted to analyze the relationship between different properties of tree species.

RESULTS AND DISCUSSION

The saplings of oak and beech trees exhibited different morphological, anatomical, and mechanical results in their stems.

Stem Morphological Characteristics

Growth and morphology are important traits of forest trees to show growth performance and survival to environmental factors (temperature, light efficiency, water, length of the growing season, soil type, *etc*.). To survive, many tree species adapt to environmental and climatic factors differently. Trees show different growth performance even in similar age and similar habitat.

Table 1 shows the morphological traits (stem diameter, node numbers, pith%, bark%, wood%), anatomical traits (average annual ring width, the number of wood ray per mm^2, ray length, ray width), and wood density results of two different tree species in a whole-stem basis. In this study, the mean stem diameter was 9.6 mm in oak stems and 9.2 mm in beech stems. However, no significant difference was found in stem diameter between the two species (oak *vs*. beech) ($p > 0.05$).

The pith radius, pith area (%), bark area (%), wood area (%), and node numbers differed significantly between two species ($p < 0.05$), and each species showed different patterns in their stems. Pith radius, pith%, bark%, and node number were greater in oak stems than beech stems. Oak stems had almost 1.5 times higher pith radius and pith% than beech stems. Pith is an important part of the stem because it is located in the central region of cross-section and made up of soft parenchyma cells that provide nutrient storage. Therefore, the function of pith is to transport stored minerals and nutrients throughout the plant. These results suggest that greater pith radius and area provided better mechanisms to use soil resources efficiently for providing a high rate of plant growth and stem stability.

Table 1. Differences in Mean Morphological, Anatomical, and Wood Density Variables of Two Species (Oak *vs.* Beech)

	Means for species[b]	
Variables[a] (Morphological, Anatomical and Physical Traits)	**Oak**	**Beech**
Over bark stem diameter (mm)	9.6a	9.2a
Node number per length	16.1a	11.1b
Pith radius (mm)	0.63a	0.44b
Pith area (%)	13.9a	9.1b
Bark area (%)	15.1a	10.1b
Wood area (%)	70.9a	80.7b
Average ring width (mm)	0.76a	0.99b
Ray numbers	12.8a	22.9b
Ray length (µm)	428.3a	309.6b
Ray width (µm)	63.5a	53.8b
Wood density (g cm^{-3})	0.53a	0.53a

[a] Variables**:** Diameter: diameter at the central point of the stem's length, Node numbers = the number of leaf nodes along the shoot length, pith radius: the radius of pith, pith% = the area of pith in the cross-section of the stem, bark% = the area of bark in the cross-section of the stem, wood% = the area of wood in the cross-section of the stem, average ring width = averaged width of the annual ring, ray numbers = ray numbers per unit area (mm^2), ray length= the length of ray, ray width: the width of ray, wood density = stem density (g cm^{-3}).
[b] Tabled means are least-squares means: means with the same letter are not significantly different ($p > 0.05$) and those with different letters are significantly different ($p \leq 0.05$) (the number of samples for morphological parameters and average ring width was 40 and for ray measurements, 25 measurements were conducted for each species).

The bark is also an important outermost layer of the plant because it protects the plants from external threats (Thomas 2001; Romero and Bolker 2008; Midgley *et al.* 2010; Hoffmann *et al.* 2012; Lawes *et al.* 2013; Ferrenberg and Mitton 2014; Pausas 2015; Rosell 2016). In this research, oak stems showed 49% greater bark area than beech stem. A previous study by Rossel (2016) showed that increasing bark thickness made stems thicker. In the same study, thicker stems also showed great properties in the fire return interval. The bark also can provide mechanical support to plant stems, since thicker bark could make stem stronger and stiffer (Niklas 1999; Rosell and Olson 2014). This study agrees with the previous study by Rossell (2016) because oak stems had greater bark area so showed thicker stems. It could be suggested that oak stems have a greater protective mechanism to external threats (*e.g.*, extreme temperature and fire) than beech stems due to their greater bark area. The wood area is also a key factor in plants because it consists of xylem tissue, which provides water and mineral transportation from roots to shoot systems for plant growth and mechanical support. In this research, beech stems surprisingly showed 13% greater wood area than oak stems ($p < 0.05$). It might be considered that beech saplings were exposed to more wind-induced bending forces and resisted the forces by increasing the xylem area.

Stem Anatomical Characteristics and Wood Density

Different tree species can show higher variability in their anatomical structure. The statistical results of wood anatomy showed that average ring width, the number of rays per mm^2 of a tangential section, ray length, and width of tangential section differed significantly between the two species (Table 1). In this study, the two tree species had different vessel arrangement: ring-porous *vs*. diffuse-porous arrangement. Oak is a ring-porous wood in which vessels with greater diameter are formed in EW and vessels with a smaller diameter in LW. Conversely, beech is a diffuse-porous tree in which vessels are distributed uniformly in size, such that vessels between EW and LW zones have the same diameter. Annual ring width is one of the important anatomical properties of wood and shows the effect of climate change on forest tree growth. The ring width is closely related to growth location, age of tree, temperature, and water and light availability. The annual ring provides information about past events that occurred in the environment. Plants with wider rings show warmer temperature and sufficient available water, and narrower rings can indicate poor growth conditions (*e.g.*, poor soil, cooler or drier weather, less water availability). The average ring width and ray number were found to be significantly greater in beech stems than oak stems. Beech stems showed almost 30% wider annual ring width (mean 0.99 mm) than oak stems (mean 0.76 mm). Previous studies suggested that when the annual ring width is narrower, the LW constitutes a greater proportion particularly in ring-porous species (Davis *et al.* 1999; Hoadley 2000). Therefore, this study suggests that beech had a higher proportion of EW cells than oak stems.

Rays were found to be mostly multiseriate and sometimes uniseriate between oak and beech trees. However, rays showed contrasting results between oak and beech. In oak, rays were 2 to 32 cells in height and 1 to 5 wide. In beech, rays were 2 to 25 cells in height and 1 to 4 cells wide. The average number of rays of the tangential section was significantly higher in beech ($p < 0.05$). The average number of rays per mm^2 of the tangential section was 11.4 in beech stems and 6.4 in oak stems. Oak stems, however, showed significantly greater multiseriate ray length and ray width values than beech stems ($p < 0.05$). The mean multiseriate ray length was 428.3 µm in oak stems and 309.6 µm in beech stems. The mean multiseriate ray width was also 63.53 µm in oak stems and 53.8 µm in beech stems (Table 1).

The results of statistical analysis showed that wood density did not differ significantly between species. Both species showed almost 0.53 g cm^{-3} wood density in their stems (Table 1). This result could be related to the study site and age of saplings because saplings were grown in the same local environments and were similar in age. Therefore, oak and beech saplings did not show significant variance in their densities.

Flexural Properties

Flexural properties varied significantly between the two species (Fig. 1). The results of statistical analysis showed that E, EI, and specific stiffness values differed significantly between oak and beech ($p < 0.05$). E, EI, and specific stiffness values were significantly greater in oak stems than beech stems ($p < 0.05$). E is a measurement of wood stiffness that demonstrates wood resistance to bending stresses. The average E was highest in oak stems with a mean of 119.9 MPa and was 78.4 MPa in beech stems; oak stems were more than 1.5 times stiffer than beech stems. However, EI is an ecologically more important parameter than E because it is mainly affected by stem radius, therefore determining the overall mechanical response of the stem to environmental loading. The average EI was 1.11 Nm^2 in oak stems and 0.62 Nm^2 in beech stems; the oak stems thus were more than 1.7 times stronger than beech stems (Fig. 1). Specific stiffness or specific E also measures the ratio of E to per unit weight and is an important parameter for stem stability. Similarly, oak showed 1.6 times (mean 224.1 MPa/g cm^{-3}) greater specific stiffness than beech (mean 143.6 MPa/g cm^{-3}). Greater specific stiffness means wood is stiffer per unit weight (Nocetti *et al.* 2010; Weber *et al.* 2017). The results of the statistical analysis, however, indicated that there was no significant difference in M_{max} and *max. long. stress* values in two species ($p < 0.05$). Mean M_{max} was almost 1.1 times greater in oak stems than beech stems (mean 4.36 Nm in oak and 4.14 Nm in beech). *Max. long. stress* showed quite similar values between oak (47.7 Nmm^2) and beech stems (47.2 Nmm^2) (Fig. 1). The oak stems were stiffer and stronger than beech stems, suggesting that the plant stem stability and survival adaptations were better in oak stems than beech stems.

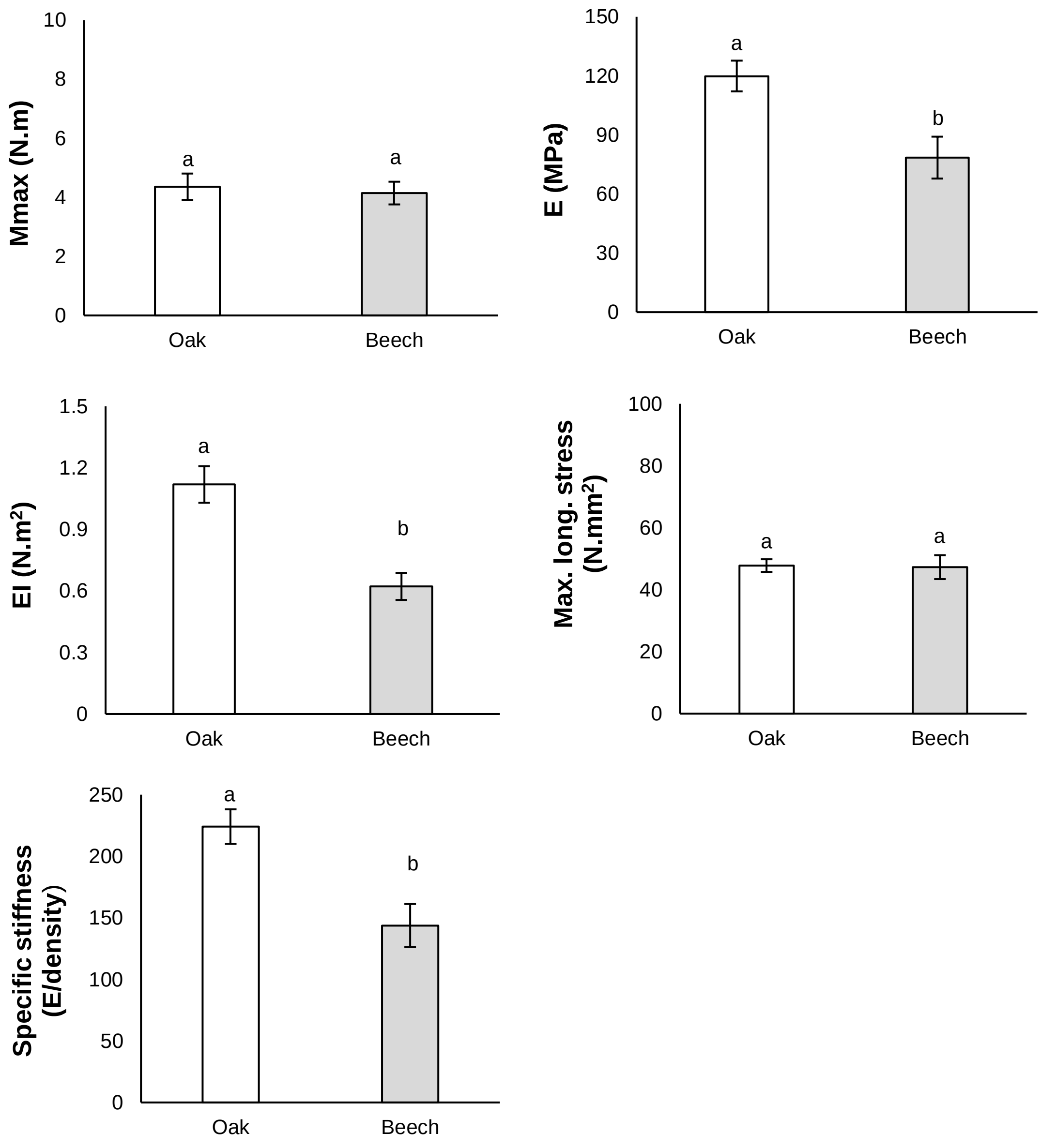

Fig. 1. Mean bending strength (M_{max}) values, flexural modulus (E) values, flexural stiffness (EI) values, maximum longitudinal bending stress (*max. long. stress*) values, and specific stiffness (E/density) values in two tree species (oak and beech). The standard error is shown by the error bars. Results of one-way ANOVA tests are denoted using letters; columns labeled with the same letter present no significant difference and values with different letters show a statistically significant difference at $p < 0.05$.

The effect of morphological and anatomical characteristics on E, EI, and *specific stiffness* values was assessed by the linear regression analysis. The node number, pith radius, pith%, bark%, wood%, average ring width, ray numbers, ray length, and ray width were used as predictor variables to explain the differences in flexural properties between oak and beech. Previous studies found that the mechanical properties of wood are negatively affected by the increase in node numbers (Bergman *et al.* 2009; Caringella *et al.* 2014; Özden and Ennos 2018). In this study, the node number was significantly greater in oak stems (mean 16.1) than the beech (mean 11.1) stems. However, linear regression analysis did not find a significant relationship between *E* and the node numbers, between *EI* and node numbers, and similarly between *specific stiffness* and node numbers. The results of the regression analysis also revealed that there was no relationship between average ring width and flexural properties (*E, EI,* and *specific stiffness*).

Conversely, regression analysis showed that pith radius and bark% had a significantly positive correlation with the *E* values that is *E* increased with increasing pith radius and bark % (R^2 = 0.12 for pith% and R^2 = 0.10 for bark%, $p < 0.05$). In the present study, oak saplings showed significantly higher pith radius and bark% than beech stems and so higher *E* values. Similarly, regression analysis also found positive significant relationships between pith% and *EI* ($R^2 = 0.24$ $p < 0.05$) and bark% and *EI* ($R^2 = 0.15$ $p < 0.05$). However, ray numbers and wood% had significantly negative correlation with the *EI* ($R^2 = 0.32$ and $R^2 = 0.22$, $p < 0.05$). Furthermore, regression analysis found that there were significant positive relationships between pith% and *specific stiffness* and bark% and *specific stiffness* ($R^2 = 0.13$, $p < 0.05$), pith% and *E* ($R^2 = 0.11$, $p < 0.05$). Similarly, wood% had negative effect on the *specific stiffness* values ($R^2 = 0.11$, $p < 0.05$). Previous studies also revealed similar results in coppice stems of different tree species. In coppice stems, there is a positive significant relationship between pith% and *E* values which means that more pith% provided greater *E* values. More pith could enable extension growth quickly so coppice stems could show better mechanical properties (Mosbrugger 1990; Niklas 1992; Briand *et al.* 1999; Özden and Ennos 2018). Sapling and coppice stems show quite similar growth adaptation because both stems tend to grow upright and use soil resources (nutrients and water) directly (Özden and Ennos 2018).

CONCLUSIONS

1. The morphological and anatomical properties of saplings showed great variation between two different tree species. Oak saplings showed greater stem diameter, node number, pith radius, pith%, bark%, multiseriate ray length, and width than beech stems. However, beech stems showed higher average ring width, wood%, and ray numbers.
2. Flexural properties (*E*, *EI*, and *specific stiffness*) were greater in oak stems than beech stems. The flexural properties were positively correlated to the stem morphology. Oak had greater pith radius, pith%, and bark% and so showed greater *E*, *EI*, and *specific stiffness* values. However, no relationship was found between wood anatomy and flexural properties.
3. This study indicated that different tree species grown in a similar environment and a similar age had different growth properties and tree stability. The research suggests that oak stems have a good adaptation to the environments at the sapling stage because oak saplings revealed better growth variation and tree stability than beech saplings.

REFERENCES CITED

Anten, N. P. R., von Wettberg, E. J., Pawlowski, M., and Huber, H. (2009). "Interactive effects of spectral shading and mechanical stress on the expression and costs of shade avoidance," *The American Naturalist* 173, 241-255. DOI: 10.1086/595761

Ashby, M. F., Easterling, K. E., Harrysson, R., and Maiti, S. K. (1985). "The fracture and toughness of woods," *Proceedings of the Royal Society of London* 398(1815), 261-280. DOI: 10.1098/rspa.1985.0034

Badel, E., Ewers, F. W., Cochard, H., and Telewski, F. W. (2015). "Acclimation of mechanical and hydraulic functions in trees: Impact of the thigmomorphogenetic process," *Frontiers in Plant Science* 6, 266. DOI: 10.3389/fpls.2015.00266

Barnett, J., and Jeronimidis, G. (2003). *Wood Quality and Its Biological Basis*, Blackwell, Hoboken, NJ, USA.

Beeckman, H. (2016). "Wood anatomy and trait-based ecology," *IAWA Journal* 37(2), 127-151

Bergman, B. A., Ewers, F. W., and Bobich, E. (2009). "Effect of leaf nodes on the mechanical properties of stems," in: Botany and Mycology *2009*, Snowbird, UT, USA, Abstract ID 134. (http://2009.botanyconference.org/engine/search/index.php?func=detail&aid=134).

Briand, C. H., Campion, S. M., Dzambo, D. A., and Wilson, K. A. (1999). "Biomechanical properties of the trunk of the devil's walking stick (*Aralia spinosa*, Araliaceae) during the crown-building phase: Implications for tree architecture," *American Journal of Botany* 86, 1677-1682.

Burgert, I. (2006). "Exploring the micromechanical design of plant cell walls," *American Journal of Botany* 93(10), 1391-1401. DOI: 10.3732/ajb.93.10.1391

Bruchert, F., and Gardiner, B. (2006). "The effect of wind exposure on the tree aerial architecture and biomechanics of Sitka spruce (*Picea sitchensis*, Pinaceae)," *American Journal of Botany* 93(10), 1512-1521. DOI: 0.3732/ajb.93.10.1512

Caringella, M. A., Bergman, B. A., Stanfield, R. C., Ewers, M. M., Bobich, E. G., and Ewers, F. W. (2014). "Effects of phyllotaxy on biomechanical properties of stems of *Cercis occidentalis* (Fabaceae)," *American Journal of Botany* 101(1), 206-210. DOI: 10.3732/ajb.1300260

Dahle, G. A., and Grabosky, J. C. (2010). "Allometric patterns in *Acer platanoides* (Aceraceae) branches," *Trees Structure and Function* 24, 321-326. DOI: 10.1007/s00468-009-0401-5

Dai, W. (2019). *Stress Physiology of Woody Plants*, CRC Press, New York, NY, USA.

Eloy, C., Fournier, M., Lacointe, A., and Moulia, B. (2017). "Wind loads and competition for light sculpt trees into self-similar structures," *Nature Communications* 8, 1014. DOI: 10.1038/s41467-017-00995-6

Ennos, A. R. (1993). "The scaling of root anchorage," *Journal of Theoretical Biology* 161, 61-75

Ennos, A. R. (2012). *Solid Biomechanics*, Princeton University Press, Princeton, NJ, USA.

Ennos, A. R. (1997). "Wind as an ecological factor," *Trends in Ecology & Evolution* 12, 108-111. DOI: 10.1016/S0169-5347(96)10066-5

Ennos, A. R. (2001). *Trees*, The Natural History Museum, London, UK.

Eugster, W. (2008). "Wind effects," in: *Ecological Processes* (Vol. 5 of *Encyclopedia of Ecology*), S. E. Jorgensen and B. D. Fath (eds.), Elsevier, Oxford, UK.

Fisher, J. B., and Honda, H. (1979a). "Branch geometry and effective leaf area: A study of Terminalia-branching pattern. I. Theoretical trees," *American Journal of Botany* 66(6), 633-644

Fisher, J. B., and Honda, H. (1979b). "Branch geometry and effective leaf area: A study of Terminalia-branching pattern. 2. Theoretical trees," *Ibid* 66(6), 645-655

Gardiner, B., Berry, P., and Moulia, B. (2016). "Review: Wind impacts on plant growth, mechanics and damage," *Plant Science* 245, 94-118. DOI: 10.1016/j.plantsci.2016.01.006

Genet, A., Auty, D., Achim, A., Bernier, M., Pothier, D., and Cogliastro, A. (2013). "Consequences of faster growth for wood density in northern red oak (*Quercus rubra* Liebl.)," *Forestry* 86(1), 99-110. DOI: 10.1093/forestry/cps057

Gibson, L. J., Ashby, M. F., and Easterling, K. E. (1988). "Structure and mechanics of the iris leaf," *Journal of Materials Science* 23, 3041-3048. DOI: 10.1007/BF00551271

Gibson, L. J. (2012). "The hierarchial structure and mechanics of plant materials," *Journal of the Royal Society Interface* 9, 2749-2766. DOI: 10.1098/rsif.2012.0341

Haberlandt, G. (1928). *Physiological Plant Anatomy*, MacMillian and Co., London, UK.

Harper, J. L. (1977). *Population Biology of Plants*, Academic Press, London, UK.

Haghshenas, M., Mohadjer, M. R. M., Attarod, P., Pourtahmasi, K., Feldhaus, J., and Sadeghi, S. M. M. (2016). "Climate effect on tree-ring widths of *Fagus orientalis* in the Caspian forests, northern Iran," *Forest Science and Technology* 12(4), 176-182. DOI: 10.1080/21580103.2016.1144542

Hoadley, R. B. (2000). *Understanding Wood: A Craftsman's Guide to Wood Technology*, The Taunton Press Inc., Newtown, USA.

Ifju, G., and Kennedy R. W. (1962). "Some variables affecting microtensile strength of Douglas-fir," *Forest Products Journal* 12, 213-217

Kitajima, K., and Fenner, M. (2000). "Ecology of sapling regeneration," in: *Seeds: The Ecology of Regeneration in Plant Communities*, M. Fenner (ed.), CAB Publishing, London, UK.

Lambers, H., and Chapin, F. S. (1998). *Plant physiological ecology*, Springer, New York, USA.

MacDonald, E., and Hubert, J. (2002). "A review of the effects of silviculture on timber quality of Sitka spruce," *Forestry* 75(2), 107-138. DOI: 10.1093/forestry/75.2.107

Mosbrugger, V. (1990). *The Tree Habit in Land Plants*, Springer, Berlin, Germany.

Nagashima, H., and Hikosaka, K. (2011). "Plants in a crowded stand regulate their height growth so as to maintain similar heights to neighbours even when they have potential advantages in height growth," *Annals of Botany* 108(1), 2017-214. DOI: 10.1093/aob/mcr109

Niklas, K. J. (1992). *Plant Biomechanics: An Engineering Approach to Plant Form and Function*, University of Chicago Press, Chicago, USA.

Niklas, K. J. (1997a). "Mechanical properties of black locust (*Robinia pseudoacacia* L.) wood: Size- and age-dependent variations in sap and heartwood," *Annals of Botany* 79(3), 265-272. DOI: 10.1006/anbo.1996.0340

Niklas, K. J. (1997b). “Size- and age-dependent variations in the properties of sap- and heartwood in black locust (*Robinia pseudoacacia* L.),” *Annals of Botany* 79(5), 473-478. DOI: 10..1006/anbo/79.5.473
Niklas, K. J. (1997c). “Mechanical properties of black locust (*Robinia pseudoacacia* L.) wood: correlations among elastic and rupture moduli, proportional limit, and tissue density and specific gravity,” *Annals of Botany* 79(5), 479-485. DOI: 10.1006/anbo/79.5.479
Niklas K. J. (1997d). “Responses of hollow septate stems to vibrations: biomechanical evidence that nodes can act mechanically as spring-like joints,” *Annals of Botany* 80(4), 437-448. DOI: 10.1006/anbo.1997.0463
Niklas, K. J., and Spatz, H. C. (2010). “Worldwide correlations of mechanical properties and green wood density,” *American Journal of Botany* 97(10), 1587-1594. DOI: 10.3732/ajb.1000150
Niklas, K. J., and Spatz, H. C. (2000). “Wind-induced stresses in cherry trees: evidence against the hypothesis of constant stress level,” *Trees Structure and Function* 14, 230-237. DOI: 10.1007/s004680050008
Onoda, Y., Richards, A., and Westoby, M. (2010). “The relationship between stem biomechanics and wood density is modified by rainfall in 32 Australian woody plant species,” *New Phytologist* 185(2), 493- 501. DOI: 10.1111/j.1469-8137.2009.03088.x
Özden, S., Slater, D., and Ennos, A. R. (2017). “Fracture properties of green wood formed within the forks of hazel (*Corylus avellana* L.),” *Trees* 31, 903-917. DOI: 10.1007/s00468-016-1516-0
Özden, S., and Ennos, A. R. (2018). “The mechanics and morphology of branch and coppice stems in three temperate tree species,” *Trees* 32, 933- 949. DOI: 10.1007/s00468-018-1687-y.
Petterson, R. W., and Bodig, J. (1983). “Prediction of fracture toughness in conifers,” *Wood and Fiber Science* 15(4), 302-316
Poorter, L. (2001). “Light-dependent changes in biomass allocation and their importance for growth of rain forest tree species,” *Functional Ecology* 15, 113-123
Putz, F. E., Coley, P. D., Lu, K., Montalvo, A., and Aiello, A. (1983). “Uprooting and snapping of trees: structural determinants and ecological consequences,” *Canadian Journal of Forest Research* 13, 1011-1020. DOI: 10.1139/x83-133
Salleo, S., and Lo Gullo, M. A. (1986). “Xylem cavitation in nodes and internodes of whole *Chorisia insignis* H. B. et K. plants subjected to water stress: Relations between xylem conduit size and cavitation,” *Annals of Botany* 58(4), 431-441. DOI: 10.1093/annbot/58.4.431
Schulgasser, K., and Witztum, A. (1992). “On the strength, stiffness and stability of tubular plant stems and leaves,” *Journal of Theoretical Biology* 155(4), 497-515. DOI: 10.1016/S0022-5193(05)80632-0
Smith, I., and Chui, Y. H. (1994). “Factors affecting mode I fracture energy of plantation-grown red pine,” *Wood Science and Technology* 28, 147-157. DOI: 10.1007/BF00192693
Spatz, H. C., Speck, T., and Vogellehner, D. (1990). “Contributions to the biomechanics of plants. I. Stabilities of plant stems with strengthening elements of different cross sections against weight and wind forces,” *Botanica Acta* 103(1), 111-122
Spatz, H. C., Beismann, H., Emmans, A., and Speck, T. (1995). “Mechanical anisotropy and inhomogeneity in the tissues comprising the hollow stem of the giant reed *Arundo donax*,” *Biomimetics* 3, 141-155.

Spatz, H. C., Boomgaarden, C. H., and Speck, T. (1993). "Contributions to the biomechanics of plants. III. Experimental and theoretical studies of local buckling," *Botanica Acta* 106, 254-264.
Sterck, F. J., Schieving, F., Lemmens, A., and Pons, T. L. (2005). "Performance of trees in forest canopies: Explorations with a bottom-up functional-structural plant growth model," *NewPhytologist* 166, 827-843. DOI: 10.1111/j.1469-8137.2005.01342.x
Szwagrzyk, J., Szewczyk, J., and Maciejewsk, Z. (2012). "Shade-tolerant tree species from temperate forests differ in their competitive abilities: A case study from Roztocze, south-eastern Poland," *Forest Ecology and Management* 282, 28-35. DOI: 10.1016/j.foreco.2012.06.031
Telewski, F.W. (2012). "Is windswept tree growth negative thigmotropism?," *Plant Science* 184, 20-28. DOI: 10.1016/j.plantsci.2011.12.001
Thomas, D. S., Montagu, K. D., and Conroy, J. P. (2007). "Temperature effects on wood anatomy, wood density, photosynthesis and biomass partitioning of *Eucalyptus grandis* saplings," *Tree Physiology* 27(2), 251-260. DOI: 10.1093/treephys/27.2.251
Thomas, P. (2000). *Trees: Their Natural History*, Cambridge University Press, Cambridge, UK.
Tyree, M. T., and Zimmermann M. H. (2002). *Xylem Structure and the Ascent of Sap*, Springer, Berlin, Germany.
Utsumi, Y., Bobich, E. G., and Ewers F. W. (2010). "Photosynthetic, hydraulic and biomechanical responses of *Juglans californica* shoots to wildfire," *Oecologia* 164(2), 331-338. DOI: 10.1007/s00442-010-1653-x
Vincent, J. F. V. (1992). *Biomechanics Materials: A Practical Approach*, Oxford University Press, Oxford, UK.
Vollsinger, S., Mitchell, S. J., Byrne, K. E., Novak, M. D., and Rudnicki, M. (2005). "Wind tunnel measurements of crown streamlining and drag relationships for several hardwood species," *Canadian Journal of Forest Research* 35(5), 1238-1249. DOI: 10.1139/x05-051
Vranckx, G., Jacquemyn, H., Mergeay, J., Cox, K., Janssens, P., Gielen, B. A. S., Muys, B., and Honnay, O. (2014). "The effect of drought stress on heterozygosity-fitness correlations in pedunculate oak (*Quercus robur*)," *Annals of Botany* 113(6), 1057-1069. DOI: 10.1093/aob/mcu025
Wang, D., Lv, S., Jiang, P., and Li, Y. (2017). "Roles, regulation, and agricultural application of plant phosphate transporters," *Frontiers in Plant Science* 8, 817. DOI: 10.3389/fpls.2017.00817
Warren, E., Smith, G. B., Apiolaza, L. A., and Walker, J. C. F. (2009). "Effect of stocking on juvenile wood stiffness for three Eucalyptus species," *New Forests* 37, 241-250. DOI: 10.1007/s11056-008-9120-9
Weber, J. C., Montes, C. S., Garcia, R. A., Silva, D. A., and Muniz, G. I. B. (2017). "Variation in growth, wood stiffness and density, and correlations between growth and wood stiffness and density in five tree and shrub species in the Sahelian and Sudanian ecozones of Mali," *Trees* 31, 833-849. DOI: 10.1007/s00468-016-1508-0
Zimmermann, M. H. (1978a). "Hydraulic architecture of some diffuse porous trees," C*anadian Journal of Botany* 56(18), 2286-2295. DOI: 10.1139/b78-274

Zimmermann, M. H. (1978b). "Structural requirements for optimal water conduction in tree stems," in: *Tropical Trees as Living Systems*, P. B. Tomlinson and M. H. Zirnmermann (eds.), Cambridge University Press, Cambridge, UK.
Zimmermann, M. H., and Sperry, J. S. (1983). "Anatomy of the palm *Rhapis excelsa*. IX. Xylem structure of the leaf insertion," *Journal of the Arnold Arboretum* 64(4), 599-609.

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