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Joseph Kusi
East Tennessee State University

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Variations in Phenotypic Plasticity and Fluctuating Asymmetry of Leaf Morphology of Three *Quercus* (Oak) Species in Response to Environmental Factors

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A thesis
presented to
the faculty of the Department of Biological Sciences
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Masters of Science in Biological Sciences

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by
Joseph Kusi
May 2013

Dr. Istvan Karsai, Chair
Dr. Tim McDowell
Dr. Christopher Liu

Keywords: *Quercus*, leaf morphology, phenotypic plasticity, fluctuating asymmetry, GLM

ABSTRACT

Variations in Phenotypic Plasticity and Fluctuating Asymmetry of Leaf Morphology of Three *Quercus* (Oak) Species in Response to Environmental Factors

by

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Leaf morphology of *Quercus* (oak) species is highly variable and complicated confounded with phenotypic plasticity and fluctuating asymmetry (FA). However, the study of variation is mostly limited to leaf morphology. This study was extended to plasticity and FA variations in *Q. alba* (white oak), *Q. palustris* (pin oak), and *Q. velutina* (black oak). It was hypothesized that light exposure, individual trees, leaf position, and other leaf traits will influence variation in these species. Leaves were sampled from trees of these species and their morphological traits were measured. Absolute asymmetry of leaf width and area were determined and plasticity of each species was calculated. The data were analyzed using nested ANOVA with General Linear Model. Leaf morphology, plasticity and FA varied across the species and light exposure was the main source of variation. Individual trees and several leaf covariate traits also influenced leaf morphological and FA variations in all species.

DEDICATION

I dedicate this thesis to Almighty God who gives me life, strength, protection, and wisdom for all my academic achievements. I am very grateful to him, I say glory be to his name for ever and evermore. I also dedicate this work to my beloved wife Beatrice Amponsah who supported and encouraged me to make this work successful. More importantly, I dedicate this thesis to my parents Regina Afia Manu and John Kwaku Addai, farmers at Berekum-Senase in Ghana who have constantly shown keen interest and invested in my education. Finally, this work is dedicated to Lawyer Amankona Diawuo, High Court Judge at Tarkwa in Ghana, and Mr. Alex Kingsley Eshun, managing director of Western Transport, Tarkwa in Ghana, who sponsored and facilitated my trip to pursue this master's program in Biology at East Tennessee State University.

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CHAPTER 1

INTRODUCTION

Leaf Morphology of Plants

Plant morphology is considered one of the most interesting areas in plant biology and has expanded our understanding of plants growth and factors that affect their development (Ashton and Berlyn 1994; Hovenden and Vander Schoor 2004; Ponton et al. 2004; Zwieniecki et al. 2004). Plants are modular organisms which grow by repetition of parts that are highly variable under varying environmental conditions. This organ modularity could result in variation in plant morphology and plastic response (Pigliucci and Preston 2004). The hierarchical structure of branches, shoots, and leaves within the tree has been found to contribute significantly to within and among tree variation in form. The patterns of variation in leaf morphology affect organisms whose feeding and reproduction depend on leaves (Suomela and Ayres 1994). The leaf in particular has characteristic development and structure which varies across plant species (Simpson 2010). That is why Hickey (1973) found it necessary to document a classic method for detailed description of the leaf characters including the shape, margin, petiole, and venation for taxonomic, phylogenetic, and ecological studies. He observed that while some families and genera exhibit certain basic patterns of leaf structure, others vary from the basic pattern under the influence of extreme environmental conditions with possible loss of some features in some species. This claim has been confirmed by several studies using the environment as the main causative factor of variation in leaf morphology (Blue and Jensen 1988; Bruschi et al. 2003; Zwieniecki et al. 2004; Hulshof and Swenson 2010; Christianson and Niklas 2011).

The variability in leaf form has also been attributed to both genetic and environmental influences. Hovenden and Vander Schoor (2004) showed that leaf traits such as the length, width, and area are controlled by both environmental and genetic factors; however, the environmental factor has overriding influence on these traits. Changes in the interaction between these 2 factors may result in evolution of morphological traits such as shape. As evolutionary process continues, the shapes that enable the organism to function more effectively in the environment are more favored by natural selection. Different shapes can be quantified to determine their adaptive importance (Klingenberg 2010). For instance, quantifying different leaf shapes may deepen our understanding of their adaptive significance in terms of photosynthesis and transpiration as shown by Nicotra et al. (2008). In that study, more dissected and less dissected leaf shapes from *Pelargonium* species were used to compare their plastic response in different climatic conditions. They observed that more dissected leaves showed higher photosynthetic and transpiration rates at higher temperatures and concluded that evolution of dissected leaves was partly a mechanism to increase photosynthesis and water transport. Previous studies have shown evidence of genetic variation in leaf development in different plant species (Juenger et al. 2005). The leaves of a plant develop under the control of genes; thus, any occurrence of gene mutation may result in genetic variation which affects the shape and size of the phenotype. Juenger et al. (2005) used *Arabidopsis thaliana* to investigate the genetic basis of leaf characters variation and their result showed significant genetic variation for shape and size.

The relationship between leaf morphology and the factors that influence its variability has been used in addressing a wide range of biological and ecological issues: investigations of past and present climate changes (Poole et al. 1996; Gienapp et al. 2008; Gimeno et al. 2008), species identification (Jensen 1988; Hess and Stoynoff 1998), air quality (Wuytack et al. 2010), plant-herbivore interaction (Cornelissen et al. 2003; Puerta-Pinero et al. 2008), and adaptation (Ashton and Berlyn 1994; Gratani et al. 2006; Sack et al. 2006). One group of species which has gained focus of attention for research in leaf morphology is the genus *Quercus*-the oaks- due to complexity of leaf variation within and among the species (Hess and Stroynoff 1998; Bruschi et al. 2000; Gonzalez-Villarreal 2003; Nixon, 2006). In the southeastern United States, *Quercus* is recognized as one of the most difficult groups to identify to species and the most challenging for botanists developing viable dichotomous keys (Kirchoff et al. 2011).

Leaf Morphology of *Quercus*

Quercus species have high morphological variability especially in leaf morphology (Hess and Stoynoff 1998; Nixon 2006). The leaves of *Quercus* are often used for morphometric study because they are highly variable, unlike the relatively uniform reproductive organs (flowers and fruits) of these species (Penaloza-Ramirez et al. 2010). The use of leaf for morphological variation analysis can differentiate clearly between individuals of *Quercus* species (Jensen 1990). The most characteristic leaf feature of *Quercus* species in eastern North America and Europe is the lobed leaf margin. Conversely, most species in subtropical and tropical regions have entire leaves (without lobes or teeth) or regularly-toothed leaves with no lobes (Nixon 2006). Baker-Brosh and Peet (1997) observed that angiosperms growing in different habitats have evolved leaf margins suitable to the climatic conditions. They showed that the leaf margins of *Q. alba, Q. rubra* and *Q. stellata* and some other species in the temperate are lobed with smooth margins or lobed with pointed margins or toothed margins. The lobed margins of these species were found to have increased photosynthetic activity. Based on this, they suggested that the lobed margins are evolutionary adaptive features that initiate photosynthetic activity in young leaves. The leaf margin is also important in distinguishing the major groups of *Quercus*. The red/black oaks have lobes with usually bristle-tipped while the white oaks have lobes with usually round margin (Mercker et al. 2006).

Sources of Variation in Leaf Morphology

Studies of leaf trait variation in *Quercus petraea* which involved a series of hierarchical designs to determine the extent of variation at each level have shown that population, tree and leaf order levels each accounted for significant variation in leaf traits (Bruschi et al. 2000; Bruschi et al. 2003; Viscosi and Cardini 2011). These studies differ from other morphometric studies in their use of the nested ANOVA technique to determine the effects of population as a fixed factor and the use of both tree and leaf as random factors. In this way, the main effects of population and the nested effects of tree and leaf on the measured morphological characters could be determined (Bruschi et al. 2003).

Frequent hybridization occurring among *Quercus* species due to weak reproductive barriers between the species also contributes to the high level of species variation (Borazan and Borac 2003). Jensen (1988) studied the relationships among 4 oak species: *Q. facalta*, *Q. velutina*, *Q. coccinea*, and *Q. marilandica*. In that study, he used quantitative characters of leaf and fruit/bud to examine the occurrence of hybridization among these species in 3 communities. He found hybridization occurring between *Q. velutina* and *Q. coccinea* in one community but to his surprise, hybridization did not occur in the other community suggesting that there may be other factors such as environmental conditions responsible for the differences between the species of the other 2 communities.

Other studies such as Jensen (1988) linked variations in *Quercus* leaf morphology with environmental factors such as irradiance (Aston and Berlyn 1994; Balaguer et al. 2001; Gratani et al. 2006; Sack et al. 2006; Batos et al. 2010); soil water availability (Carpenter and Smith, 1975; Phelps et al. 1976; Dickson and Tomlinson 1996); carbon dioxide (Garcia-Amorena et al. 2006); nutrients (Valladares et al. 2000), and positional and seasonal effects (Blue and Jensen

1988). Sunlight is one of the most common environmental factors used by most researchers in recent times to monitor the effects of the environment on leaf development using sun leaves (leaves directly exposed to sunlight) and shade leaves (leaves indirectly exposed to sunlight) in their natural environment (Zwieniecki et al. 2004; Gratani et al. 2006; Sack et al. 2006; Batos et al. 2010; Brodribb and Jordan 2011). In all the studies investigating irradiance plasticity effects, leaf morphological, and anatomical trait variations were pronounced in sun versus shade leaves. These sun/shade differences were similar to differences among leaves from xeric versus mesic habitats respectively (Abrams 1990). Sun leaves were found to be thicker, smaller, wide, and deeply lobed, having higher stomatal density, reduced stomatal size, and small stomatal pore while shade leaves were thinner, larger, less deeply lobed with lower stomatal density and larger stomatal pore. Similar results were also found in other species such as *Alnus glutinosa* (Poole et al. 1996); Hawaiian *Plantago taxa* (Dunbar-Co et al. 2009) and *Nothofagus cunninghamii* (Brodribb and Jordan 2011).

Significance of Leaf Modification

Leaf modification within species has been suggested to enhance plants performance in different environments and resource acquisition (Castro-Diez et al. 1997). An investigation of leaf conductance of mesic versus xeric habitats in *Acer saccharum, Quercus alba, Q. rubra* and *Q. velutina* revealed that among the oaks, *Q. alba* was more tolerant to shade, *Q. velutina* was more drought tolerant and *Q. rubra* exhibited the highest stomatal density (Phelps et al. 1976). This demonstrates how different leaf morphology in these species enhanced water availability and drought tolerance. Modification of sun leaves enhances regulation of water loss through transpiration and uptake of carbon dioxide by diffusion, whereas modification of shade leaves enhances photosynthetic rates (Franks and Beerling 2009). Leaf morphology also indicates mechanisms such as fluctuating asymmetry (FA), phenotypic plasticity and adaptation used by *Quercus* to cope with environmental impacts (Ashton and Berlyn 1994; Hodar 2002; Cornelissen et al. 2003; Gratani et al. 2006; Gimeno et al. 2008; Hulshof and Swenson 2010). The common idea which unites these studies is how evolution of the varying leaf traits in combination with these mechanisms helps the plant to response appropriately to climate changes.

Fluctuating Asymmetry

Fluctuating asymmetry is the most effective variation component that can be used to control the effects of genetic and environmental factors (Klingenberg 2002). In an ideal environment, plants are expected to exhibit developmental stability, the ability of a genotype to produce a particular type of phenotype in a given environment. However, most of these plants show developmental instability as a result of coping with varying, stressful environmental conditions. Developmental instability is common in plants because they are modular organisms with repeated parts influenced by different environmental conditions. Thus, morphological asymmetric traits are likely to be observed when bilateral symmetry is used to study morphological instability in plants (Freeman et al. 1995). Fluctuating asymmetry (FA), a small, random deviation from bilateral symmetry, is the type of asymmetry used to measure developmental instability in plants (Cornelissen et al. 2003). Most studies of bilateral symmetry have shown that the presence of FA displayed by symmetric traits of an organism is an indication of response to environmental stress (Cornelissen et al. 2003; Puerta-Pinero et al. 2008; Tucic and Miljkovic 2010) and genetic stress (Pertoldi et al. 2006). Apart from this, the presence of FA has been suggested as an indicator of herbivory because asymmetric leaves are more nutritionally rich as compared to symmetric leaves and that are more susceptible to herbivores (Cornelissen et al. 2003). However, Palmer (1994) found that the data of several studies suggesting FA as a reliable indicator of both environmental and genetic stresses were inconsistent and he attributed this inconsistency to limited statistical power. Graham et al. (2010) made a constructive argument in their review of several studies on FA. Although they acknowledged FA as an indicator for both environmental and genetic stress, they provided evidence to support the claim that phenotypic plasticity is more sensitive to stress than FA. They also suggested that the FA will be more pronounced if the adaptive mechanism of the plant failed to buffer stress and in such situation FA may be the next option to compensate for the stress.

Asymmetrical leaves have been identified in some oak species: *Q. ilex* (Hodar 2002); *Q. myrtifolia* and *Q. geminata* (Cornelissen et al. 2003); *Q. laurina* and *Q. affinis* (González-Rodríguez and Oyama 2005); and *Q. magnoliifolia* and *Q. resinosa* (Albarrán-Lara et al. 2010). For instance, leaves of *Q. laurina* and *Q. affinis* showed different number of lobes and teeth on either side of most leaves midribs indicating the presence of developmental instability as a result of gene mutation (González-Rodríguez and Oyama 2005). Fluctuating asymmetry was also examined in *Q. magnoliifolia* and *Q. resinosa*; hybridization and introgression between these 2 species were found to be the main factors which had significant effects on FA (Albarrán-Lara et al. 2010). Although most of these studies examined ecological factors which influenced FA, little is known about the effects of sunlight exposure, individual trees, leaf position, and leaf morphological traits on FA. It is believed that knowing the effects of these factors will improve our understanding on developmental instability in *Quercus* species.

Phenotypic Plasticity

Phenotypic plasticity is a phenomenon by which a given genotype produces different phenotypes in different environmental conditions. Phenotypic plasticity involves the creation of appropriate match between the environment and phenotype (Pigliucci and Preston 2004; Bateson

2007). This interaction between the plant and its environment reveals patterns of phenotypic plasticity in some parts of the plant, such as the leaf which compensates for disruption in the plant development under unfavorable conditions (Sultan 2000). Schlichting (1986) proposed that the extent of variation in plasticity between taxa is partly caused by adaptation and added that selection, genetic drift, and disruption of the genetic system are the driving forces which bring about evolutionary changes in phenotypic plasticity. To overcome this limitation, plants develop adaptive strategies to minimize the detrimental effects of adverse environmental conditions (Rozendaal et al. 2006). The heterogenic nature of light causes individuals of the same species and leaves of the same tree to experience contrasting light intensity exposure (Valladares et al. 2000), and several studies have investigated phenotypic plasticity in response to variable light levels (Valladares et al. 2003; Gratani et al. 2006; Sack et al. 2006; Gimeno et al. 2008).

Plastic response to environmental drivers has been found to be higher in leaf morphology of many *Quercus* species enabling them to adapt quickly to different environmental conditions (Carpenter and Smith 1975; Phelps et al. 1976; Blue and Jensen 1988; Ashton and Berlyn 1994). Plastic response enables oak species to modify their leaf morphological and anatomical traits to cope with adverse environmental conditions. These modified traits control water transport, cooling, prevention of photochemical damage, and maintenance of minimum photosynthetic rate (Dickson and Tomlinson 1996). Phenotypic plasticity among other factors was found to have contributed to leaf variation in *Q. affinis* and *Q. laurina* (González-Rodríguez and Oyama 2005).Variation in plasticity has been found within and across canopy positions in 6 temperate deciduous trees in response to light exposure (Sack et al. 2006). For example, Ashton and Berlyn (1994) compared leaf traits of 3 *Quercu*s species; *Q. coccinea, Q. rubra, and Q. velutina*. They found *Q. velutina* exhibiting the greatest leaf anatomical plasticity and was also the most drought tolerant and light demanding species. *Quercus rubra* showed the least anatomical plasticity while *Q. coccinea* showed intermediate plasticity response. Although these studies presented evidence of plasticity occurring in *Quercus* species, they did not use the plasticity index proposed by Valladares et al. (2000) which could have further explained the existence of between species differences. A high plasticity index value is very important for high light exploitation as an adaptation for resource acquisition. It was considered an advantage to *Quercus ilex* over *Phillyrea latifolia* and *Pistacia lentiscus* in its adaptation to various environmental limitations resulting in a wider spread in diverse habitats (Gratani et al. 2006). Phenotypic plasticity also plays a significant role in ecological distribution of species (Valladares et al. 2000).

Adaptation to Changes in Environmental Conditions

Adaptation is a form of plastic response that helps an organism to adjust to different environments for successful survival. Members of a group of organisms differ in adaptive ability resulting in species diversity (Sultan 2000). Variation in leaf morphology among species at different sites is an indication that some species adapt better in some particular habitats using a different type of leaf form. For instance, small and thick leaves are adaptive to dry habitats to conserve water while large and thin leaves are adaptive to wet habitats (Rowland et al. 2001). Leaf morphology and physiology were used variously by *Quercus ilex* seedlings to cope with climate change. The leaf structure is mostly involved in local adaptation while the leaf physiology is usually used for plastic response (Gimeno et al. 2008). The lobed leaf margin of *Quercus* is believed to have evolved during the Cretaceous angiosperm radiation in response to low temperatures and since then has evolved several times making it an important adaptive trait. The lobe tip, smooth or pointed has been found to play significant role in photosynthetic activity in the juvenile leaves of *Quercus* species (Baker-Brosh and Peet 1997).

The aim of the study reported herein was to investigate variation in leaf morphology, phenotypic plasticity, and FA in response to light exposure of 3 *Quercus* (oak) species: *Quercus alba* L. (white oak), *Quercus palustris* Muench (pin oak), and *Quercus velutina* Lam. (black oak) co-occurring at the East Tennessee State University (ETSU) Arboretum. These variables were examined under different levels of sunlight exposure to determine each species response to changes in the environment. The outer versus inner positions of the tree (exterior leaves at the outer edge of the tree, versus interior leaves positioned inside the exterior leaf layer) from 2 cardinal point positions (north vs. south sides of the tree) were selected to represent different light levels. Apart from light exposure, the study also focused on the effects of individual trees within a species, leaf position on a twig within branch positions, and leaf covariate traits on leaf morphology and FA of the studied species. This study tested the following hypotheses:

H_0….there is no FA or phenotypic plasticity occurring within the species. Only random variation occurs.

H_1 ….light exposure will have a greater influence on leaf morphology, phenotypic plasticity, and FA variation within the species.

H_2 ….shade leaves will exhibit higher fluctuating asymmetry because they have limited light exposure.

H_3 ….sun leaves will exhibit greater phenotypic plasticity because they experience more severe environmental conditions.

H_4 ….individual trees, leaf position, and several leaf covariate traits will have significant influence on leaf morphology and FA variation.

CHAPTER 2

MATERIALS AND METHODS

Study Area

The study was conducted at the East Tennessee State University (ETSU) Arboretum (comprising the entire ETSU main campus) and Mountain Home Veteran Affairs (VA) Medical Center campus, Johnson City, Tennessee (See Figure 1). These sites share a common boundary and were chosen for these reasons: they have species identified by experts; they have diversity of species for research and ornamental purposes; most of the oaks at these campuses are mature trees growing in open spaces which minimize shading effects from other trees and buildings to maximize tree exposure to sunlight (Sack et al. 2006). Three oak species native to Tennessee were studied to determine variation in fluctuating asymmetry and leaf plasticity as a result of sun and shade effects.

Figure 1 Map of the sample sites showing distribution of *Quercus* species at ETSU. Bartlett Inventory Solutions-ETSU 2012.

Study Species

Quercus is the dominant genus of the family Fagaceae, which is widely distributed in the subtropical and tropical regions of the Northern Hemisphere, and extending into the Southern Hemisphere (Nixon 1993). The present wide distribution of *Quercus* is consistent with its abundant fossil record (Jay 1986). In the United States, the greatest number of *Quercus* species occurs in the southeastern region (Nixon 2006), and 20 of these species are native to Tennessee (Mercker et al. 2006). The genus *Quercus* consists of about 500 species including trees and shrubs and has 2 subgenera, *Cyclobalanopsis* and *Quercus*, commonly found in eastern Asia and North America and Europe respectively (Nixon 1993a&b; Manos et al. 1999). In the New World comprising Latin America, United States and Canada, the species number is estimated at 220 (Nixon 2006). This genus is further grouped into 4 sections: *Quercus*, *Lobatae*, *Protobalanus*, and *Cerris* (Nixon 2007). Sections *Quercus* and *Lobatae* are found in Americas, section *Cerris* is restricted to Eurasia and North Africa while section *Protobalanus* is restricted to the southeastern USA and northern Mexico. *Quercus* is the most economically important group in the family Fagaceae because of its rich species diversity, ecological dominance, and many domestic and industrial uses (Nixon 2006).

This study examined 3 species of *Quercus*: *Quercus alba* L. (white oak), *Quercus palustris* Muench (pin oak), and *Quercus velutina* Lam. (black oak). These species were chosen because of their interesting leaf margins lobes which have evolutionary adaptive significance (Baker-Brosh and Peet 1997). *Quercus alba* is considered to be the State Tree of many states in USA (Coombes 2010). The leaves are obovate to elliptic with round-tipped narrow lobes with sinuses varying from shallow to deep (See Figure 2). The emerging young leaves are pink in color and all the leaves usually turn deep red in fall. The acorns are ovoid in shape with a scaly cup and the bark has grey color. *Quercus palustris* is widely distributed in North America and is tolerant of moisture and flooding. It has drooping branches when young with leaves elliptic to oblong. The leaves have wide lobes with deep sinuses and bristle-tipped which are persistent through winter (See Figure 2). The acorns are small, dark brown, spherical in shape with a scaly cup. The bark is usually smooth and greyish-black. *Quercus velutina* is a widely distributed oak that co-occurs and hybridizes with other oak species. The leaves are elliptic to obovate with finely pubescent on the abaxial surface. The leaves are shallowly lobed with further division at the tip and bristle-tipped ends (See Figure 2). The acorns are oval born in cup covered with overlapping scales and dark-grey-brown bark. *Quercus alba* and *Q. velutina* are upland dwellers while *Q. palustris* is a bottomland dweller (Mercker et al. 2006; Coombes 2010). *Quercus alba, Q. velutina,* and *Q. palustris* are better adapted to calcareous, xeric, and mesic sites respectively (Dickson and Tomlinson 1996). All of the 3 species are deciduous, with leaves dying each winter and new replacement leaves emerging each spring.

Sampling Method

Thirty trees per species for a total of 90 trees were sampled in September and October, 2011. For each tree, the diameter at breast height (DBH) was measured with a meter rule and the height was also estimated using the formula $T_0 = R_0/R_1 \times T_1$. Where T_0 is the estimated tree height; T_1 is the length of the tree shadow; R_0 is the length of the meter rule and R_1 is the length of the meter rule shadow (Table 1). Trees were identified to species in the field using visual characteristics such as the bark, leaf shape, acorn size and shape, and pubescence of twigs and leaves (Mercker et al. 2006; Coombes 2010) and guide for species in the arboretum brochure for reference (ETSU Arboretum Species List and Map). A compass was used to detect the northern and southern facing parts of each individual tree. For the purpose of positional variability within a tree, a 4m pole cutter was used to cut branches randomly from 4 different positions of each tree (3-6m above ground) representing south outer and south inner (south-facing side of the tree) and north outer and north inner (north facing side of the tree) as indicated by the compass (Bruschi et al. 2000). Five undamaged mature leaves were collected from each branch and numbered 1-5 in order of collection for every tree position. The leaves of the tip of the twig were discarded and leaf 1 represents the first outermost remaining leaf on the branch. Subsequent numbers represent the order of leaves from that first leaf. The leaves were then arranged in 4 different envelopes (one per branch position), labeled, and sent to James Warden Herbarium, ETSU for preparation and storage. In all, a total of 1800 leaves were collected for this study (600 per species). We chose this large sample size to facilitate the study of fluctuating asymmetry (FA) on the basis of recommendation made by Graham et al. (2010). The leaves were pressed according to the Simpson (2010) method and placed in a plant drier at 70°C. After the 72hrs, the leaves were removed from the drier, arranged in their respective envelopes, and stored in a herbarium cabinet for morphometric analysis.

Table 1 Descriptive statistics of estimated tree height and diameter of *Q. alba*, *Q. palustris*, and *Q. velutina*

Trait		Height (cm)				Diameter (cm)			
Species	N	Minimum	Maximum	Mean	SE	Minimum	Maximum	Mean	SE
Q. alba	30	900.0	2800.0	1636.0	98.3	36.0	125.0	69.2	4.5
Q. palustris	30	600.0	2480.0	1581.3	73.8	23.0	120.0	70.0	3.5
Q. velutina	30	800.0	2860.0	1832.3	108.7	17.0	130.0	65.8	5.8

Morphological Traits Measurements

Images of the flattened leaves were obtained using a digital camera (Canon EOS 60D) for measurements of morphological traits. All images were obtained in the same orientation in abaxial view to ensure flatness of the leaves and easy identification of the secondary veins. For each leaf, 15 traits were measured from the digital image using ImageJ software (National Institute of Health, USA). Every leaf was photographed with a ruler and the ruler was used for ImageJ calibration for each leaf (See Figure 3). Asymmetry of the leaf width was determined by measuring the width of both right and left sides from the midrib to the widest point of the leaf. The asymmetry of the leaf area was also measured from the midrid to the margins for each side of the leaf. The following 15 morphological traits were selected because they vary in response to environmental and genetic factors (Blue and Jensen 1988; Jensen 1990; Borazan and Babac 2003). The secondary veins were determined based on the relative size of their point of origin according to Hickey (1973).

Morphological Traits Measured

M1	Petiole length (a-b)
M2	Interval between the leaf base and the largest secondary vein (b-e)
M3	Interval between the largest secondary vein and the apex (e-o)
M4	Interval between the 2 largest secondary veins (e-k)
M5	Length of the largest secondary vein (e-h)
M6	Maximal blade width of the left half (k-h)
M7	Maximal blade width of the right half (k-l)
M8	Interval between basal pairs of sinuses (c-d)
M9	Interval between apical pairs of sinuses (m-n)

M10	Sinus depth next to M5 (f-i)
M11	Sinus width nest to M5 (g-j)
M12	Area of the left half of the leaf blade
M13	Area of the right half of the leaf blade
M14	Area of leaf envelope (parameter) of the left half of the leaf blade
M15	Area of leaf envelope of the right half of the leaf blade

Calculated Traits

TLL	Total leaf length (M1 + M2 + M3)
LBL	Leaf blade length (M2 + M3)
TLW	Total leaf width (M6 + M7)
TLA	Total leaf area (M12 + M13)
TLE	Total leaf envelope (M14 + M15)
Lobulation	Total leaf envelope (TLE) minus total leaf area (TLA)
Leaf shape	Ratio of total leaf width (TLW) to total leaf length (TLL)

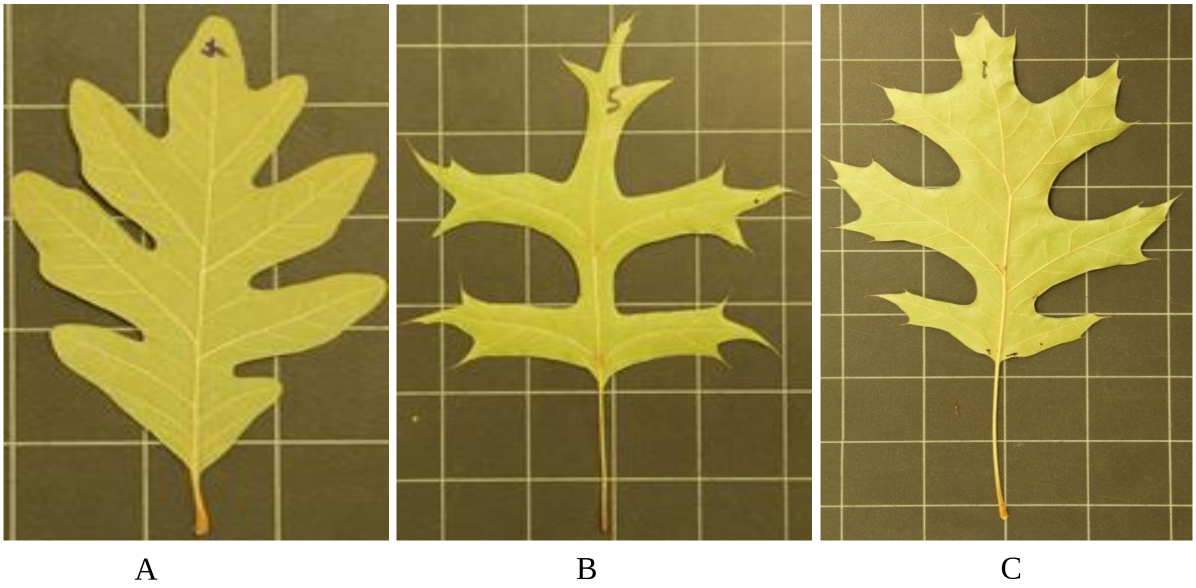

Figure 2 Leaf images representing the 3 species. A, *Q. alba*; B, *Q. palustris*, and C, *Q. velutina*

Figure 3 A leaf image of *Q. alba* showing the traits used for morphological measurements.

Statistical Analyses

Measurement of FA

According to Dongen (2006) and references therein, FA can be confounded with measurement error (ME); therefore, the first step in FA analysis is to determine the ME by repeated measurement of at least part of the dataset. Three months after completing the original measurements of all traits, 150 leaves (50 leaves per species) were randomly selected for repeated measurements. Photographs of these leaves were retaken and the leaf width as well as the area of both sides of the lamina was remeasured to ensure accuracy of the measurements. The new measurements and the old measurements were compared according to Hodar (2002) method. It is well known that measurement error is inevitable in determination of FA; however, the FA must be greater than the measurement error to make sure the symmetry differences measured is as a result of FA before any further analysis can be performed (Hodar 2002; Puerta-Pinero et al. 2008; Tucic and Miljkovic 2010). Apart from FA, there are 2 other asymmetries, directional asymmetry (DA) and antisymmetry (AS) which absence must be checked to make sure FA is the only source of deviation from bilateral symmetry for both traits (Palmer and Strobeck 1986). The DA is a deviation from bilateral symmetry where one side is usually larger than the other side and the signed mean of R-L is different from zero while AS is a bilateral variation between the sides of an individual where the larger part does not always occur at one side (Van Valen 1962; Palmer 1994). The second step is to check the normal distribution of signed asymmetry to make sure its average is not significantly different from zero. This test also detects the presence or the absence of directional asymmetry (DA) and antisymmetry (AS). If the mean of signed asymmetry was different from zero mean, DA is assumed to be present or if the data showed bimodal or platycurtic distribution, AS is also assumed to be present.

A one-sample t-test was used to test for the presence of DA in all the species by comparing the average of signed R-L value to a mean value of zero to determine whether they were significantly different. Antisymmetry was tested by examining the histograms of signed R-L to determine whether any of the traits exhibited bimodal distribution about the mean (Van Valen 1962; Tucic and Miljkovic 2010). All these tests were conducted to make sure that samples were normally distributed in the populations they represent. Before the traits could be used for further analysis, they must be tested for normality to ensure that the data are normally distributed in the populations (María-José et al. 2004; Cornelissen and Stiling 2005). All the variables measured were subjected to Kolmogorov-Smirnov test for normality and those which failed to show normal distribution were normalized by log-transformation. However, variables which failed Kolmogorov-Smirnov test but whose histograms showed normal distribution were not transformed. Absolute asymmetry width was determined by finding the absolute difference between the left width and the right width (FA =|RW-LW|). Absolute asymmetry area was also determined in the same way by finding the absolute difference between the left area and the right area (FA =|RA-LA|). The absolute value indicates the variance in FA devoid of directional asymmetry (Cornelissen et al. 2003; Graham et al. 2010). All statistical analyses were performed using Statistical Package for the Social Sciences software (SPSS 19).

Fluctuating Asymmetry and Plasticity Indices

Positive and negative size scaling are common problems associated with bilateral asymmetry and if not detected can lead to significant effects on determinations of asymmetry. This situation becomes relevant when asymmetry becomes size dependent as a result of active growth (Graham et al. 2010). According to Komogorov-Smirnov test, leaf width for all the species was normally distributed. In contrast, leaf area for all the species failed to show normal distribution and was subsequently log-transformed. A careful observation of the histograms obtained from computation of unsigned L-R for both leaf traits exhibited clear positive scaling indicating evidence of size dependence. To eliminate size dependence, the FA2 index (mean [|R-L|/((R+L)/2)]) was used to calculate FA for both leaf width and area for further analysis as recommended by Palmer (1994) and Graham et al. (2010). The calculated FA for leaf width and area failed to show normal distribution and were normalized by square root transformation. Plasticity index was calculated for each variable and species as the absolute difference between the minimum and the maximum mean values according to Valladares et al. (2000).

Models to Determine the Sources of FA Variation

A nested analysis of variance (ANOVA) was performed with General Linear Model (GLM) to determine the factors that influence FA, leaf area, lobulation, and leaf shape variations in each of the 3 species. Initial models were analyzed and all non-significant terms were removed. Then the model was reanalyzed with the significant terms and any new non-significant covariate terms were eliminated step by step until only significant terms were left in the model. Tree (random effect), position, and leaf (factors) were kept in the model until the final model was obtained. Separate models were developed for each species following the same procedure. This process (statistical elimination) continued until the residuals of the analyses indicated no discernible pattern.

The analysis followed the initial models:

$$FA_{width} = T + P + L\ (P) + M1 \ldots + M15 + \varepsilon. \tag{1}$$

$$FA_{area} = T + P + L\ (P) + M1 \ldots + M15 + \varepsilon. \tag{2}$$

$$TLA = T + P + L\ (P) + M1 \ldots + M15 + \varepsilon. \tag{3}$$

$$Lobulation = T + P + L\ (P) + M1 \ldots + M15 + \varepsilon. \tag{4}$$

$$\text{Leaf shape} = T + P + L(P) + M1\ldots + M15 + \mathcal{E}. \quad (5)$$

These models were used to test the main effects of tree and branch position, nested effect of leaf position within branch position, and effects of covariates within the species on FA. No significant interaction between tree and position was found, thus, interaction was subsequently eliminated from the model. Tree (T) was considered as random factor because trees were randomly sampled to represent the species. Position (P) and leaf (L) were considered as fixed factors while all the measured traits were considered as covariates. Leaf is commonly considered as a random factor (Bruschi et al. 2003; Gonzalez-Rodriguez and Oyama 2005), but it was considered as a fixed factor in our study because the first 5 mature leaves were selected from every branch according to our experimental design (non-random design). Several models were developed to predict the extent of FA variation for each species and those with very good residuals were finally selected through statistical elimination of covariates which were not significant. Standard residuals were plotted against unstandardized residuals to test the fitness of the model. Because we examined leaves from 4 different positions, Bonferroni pairwise comparisons test was performed to determine which pairs of means were significantly different.

CHAPTER 3

RESULTS

Measurement Error and Normality Test

The absolute bilateral differences for the 150 leaves (50 per species) measured for leaf width ranged from 0-19.57mm (mean ± SE, 4.75 ± 0.32mm), while the maximum absolute difference between the original and repeated measures was 2.00mm for the left width (0.71 ± 0.04mm) and 1.94mm for the right width (0.73 ± 0.04mm) (Table 2). The range in absolute bilateral differences between repeated measurements for leaf area was 0-1908.49mm^2 (333.16 ± 28.06mm^2), while the maximum absolute difference between the original and repeated measures was 89.93mm^2 for left area (36.79 ± 1.84mm^2) and 94.80mm^2 for the right area (35.67 ± 1.85mm^2). In order to determine the relationship between the original and repeated measures, a Pearson correlation was conducted for both traits (Table 2). This resulted in $r^2 = 0.997$, $p < 0.001$ for left width, $r^2 = 0.998$, $p < 0.001$ for right width while both left and right leaf areas had $r^2 = 1.00$, $p < 0.001$. These results depict low measurement error for both traits as compared to the FA rendering the measurements sufficiently reliable for further analysis. The test for normality using one-sample t-test (Table 3) indicated that the means of signed asymmetry (L-R) for all the species were not significantly different from zero (all *p*-values > 0.05; N = 600 per species) except FA_{area} for *Q. velutina* which was marginally significant ($p = 0.041$; N = 600). This test ruled out the presence of directional asymmetry (DA) as the cause of asymmetry in the species. Further tests failed to detect the presence of antisymmetry (AS) as none of the histograms showed bimodal distribution about the mean when examined (See Appendix Figure 12). In this regard, we were convinced that the source of bilateral asymmetry measured for both leaf width and leaf area in the population could be attributed to the presence of FA. This finding opposes hypothesis 1 of this study that there is no other type of variation occurring within the species except random variation. The boxplots for measured traits of *Q. alba*, *Q. palustris*, and *Q.velutina* revealed that no trait exhibited extreme outliers (See Appendix Figures 13-20). Most of the measured variables were normally distributed (Kolmogorov-Smirnov test; $p > 0.05$; N = 600 leaves per species) and those which failed the test were log-transformed (See Appendix Table 9). Those variables which were log-transformed include M8, M9, M12, M13, M14, M15, and TLA for all the species. For *Q. palustris*, M2 and M3 were also log-transformed to ensure normal distribution.

Table 2 The mean and standard error of original and repeated measurements to determine measurement error.

Trait	Minimum	Maximum	Mean ± SE	r^2	*p*
LW_2-RW_2	0.00	19.57	4.75 ± 0.32		
LW_1-LW_2	0.01	2.00	0.71 ± 0.04	0.997	< 0.001
RW_1-RW_2	0.02	1.94	0.73 ± 0.04	0.998	< 0.001
LA_2-RA_2	0.00	1908.49	333.16 ± 28.06		
LA_1-LA_2	0.57	89.93	36.79 ± 1.84	1.000	< 0.001
RA_1-RA_2	0.61	94.80	35.67 ± 1.85	1.000	< 0.001

LW_1 = original measurement of the left side width, LW_2 = repeated measurement of the left width, RW_1 = original measurement of the right side width, RW_2 = repeated measurement of the right side width, LA_1 = original measurement of the left side area, LA_2 = repeated measurement of the left side area, RW_1 = original measurement of the right side area and RW_2 = repeated measurement of the right side area.

Table 3 One-sample t-test to check normal distribution of FA

	Q. alba			*Q. palustris*			*Q. velutina*		
Type of FA	t	df	*p*	t	df	*p*	t	df	*p*
FA_{width}	1.389	599	0.165	0.444	599	0.657	0.013	599	0.990
FA_{area}	-0.902	599	0.368	-1.065	599	0.287	-2.053	599	0.041

Morphological Traits of *Q. alba*, *Q. palustris*, and Q. *velutina*

The means of the petiole length (M1), sinus depth (M10), and sinus width (M11) showed similar trend within the tree canopy of *Q. alba* (Table 4). The outer leaves of this species had longer petiole and deeper and wider sinuses. On the other hand, in all the remaining traits, the means of the measurements were larger in the inner leaves with the largest mean occurring at the north inner position which was observed to be the portion that experienced deep shade within the canopy.

Table 4 Morphological traits of south and north facing leaves of *Q. alba* showing their means and standard error (N = 600).

Traits	South outer	South inner	North outer	North inner
M1	16.91 ± 0.33	14.42 ± 0.37	17.35 ± 0.38	14.53 ± 0.37
M2	40.52 ± 0.68	49.08 ± 0.93	43.88 ± 0.84	49.94 ± 0.94
M3	88.74 ± 1.12	96.23 ± 1.61	94.73 ± 1.30	98.53 ± 1.56
M4	23.63 ± 0.45	25.77 ± 0.52	26.58 ± 0.54	27.62 ± 0.5
M5	58.74 ± 0.85	64.56 ± 1.10	62.47 ± 1.02	66.00 ± 1.12
M6	45.04 ± 0.66	48.90 ± 0.86	47.36 ± 0.75	49.64 ± 0.87

Table 4 Continued

M7	44.14 ± 0.61	48.55 ± 0.99	46.93 ± 0.77	49.68 ± 0.89
M8	22.45 ± 0.47	29.84 ± 0.54	24.25 ± 0.52	31.93 ± 0.62
M9	13.99 ± 0.34	17.64 ± 0.35	14.85 ± 0.33	18.97 ± 0.42
M10	36.37 ± 0.84	32.54 ± 0.95	37.35 ± 1.00	32.01 ± 0.90
M11	13.34 ± 0.43	12.70 ± 0.36	14.08 ± 0.41	12.19 ± 0.38
M12	2492.32 ± 54.41	3419.94 ± 100.20	2809.69 ± 60.52	3597.74 ± 94.80
M13	2458.36 ± 51.78	3411.66 ± 105.48	2825.09 ± 64.69	3700.44 ± 102.80
M14	3627.86 ± 79.78	4506.45 ± 134.12	4086.00 ± 94.93	4664.09 ± 126.37
M15	3583.00 ± 80.28	4522.33 ± 146.24	4114.49 ± 100.05	4747.94 ± 130.28
TLA	4950.68 ± 102.12	6831.60 ± 201.45	5634.79 ± 121.65	7298.18 ±190.78
LBL	129.26 ± 1.43	145.31 ± 2.17	138.61 ± 1.73	148.47 ± 2.08
TLL	146.17 ± 1.62	159.73 ± 2.38	155.96 ± 1.93	163.00 ± 2.26

All the measured and calculated traits followed a common pattern in *Q. palustris*. The means of the traits were greater in the inner leaves than the outer leaves (Table 5). Again, all the traits of the leaves from north inner position always showed the greatest means except petiole length (M1), maximal blade width of the left half (M6), and area of leaf blade of the left half (M12) which greatest means were found in the south inner leaves. Shorter petiole length, shallow sinus depth, and narrow sinus width in the outer leaves observed in *Q. palustris* are contrary to the usual characteristics of sun leaves exhibited by *Q. alba* and *Q. velutina* in our study (Tables 4 and 6 respectively).

Table 5 Morphological traits of south and north facing leaves of *Q. palustris* showing their means and standard error (N = 600).

Traits	South outer	South inner	North outer	North inner
M1	40.89 ± 0.63	47.99 ± 0.82	45.38 ± 0.77	47.54 ± 0.81
M2	29.81 ± 0.60	38.35 ± 0.69	30.48 ± 0.47	38.93 ± 0.72
M3	78.83 ± 1.09	87.94 ± 1.19	83.69 ± 1.21	88.57 ± 1.23
M4	25.82 ± 0.45	29.64 ± 0.47	26.98 ± 0.41	30.20 ± 0.45
M5	62.47 ± 0.76	71.70 ± 0.91	67.77 ± 0.83	71.91 ± 0.87
M6	54.93 ± 0.77	60.60 ± 0.80	60.06 ± 0.88	60.19 ± 0.83
M7	54.65 ± 0.75	60.47 ± 0.83	59.79 ± 0.83	60.53 ± 0.78
M8	13.30 ± 0.33	18.88 ± 0.47	13.73 ± 0.35	18.99 ± 0.44
M9	8.73 ± 0.15	11.05 ± 0.25	9.28 ± 0.17	11.33 ± 0.27
M10	36.71 ± 0.77	41.31 ± 0.67	40.49 ± 0.73	41.35 ± 0.74
M11	21.82 ± 0.53	22.67 ± 0.43	22.40 ± 0.53	23.51 ± 0.52
M12	1762.63 ± 39.35	2798.83 ± 77.07	2057.74 ± 48.40	2796.13 ± 61.70
M13	1767.11 ± 36.34	2806.37 ± 75.76	2087.75 ± 48.56	2824.49 ± 63.46
M14	3976.97 ± 93.48	5364.47 ± 130.19	4647.09 ± 115.93	5373.98 ± 120.16
M15	3892.56 ± 89.88	5275.78 ± 128.35	4616.95 ± 114.40	5353.75 ± 122.72
TLA	3529.74 ± 73.84	5605.20 ± 149.96	4145.50 ± 94.42	5620.62 ± 121.52
LBL	108.64 ± 1.37	126.29 ± 1.45	114.17 ± 1.36	127.50 ± 1.44
TLL	149.53 ± 1.61	174.29 ± 1.83	159.55 ± 1.91	175.04 ± 1.95

The means of petiole length (M1), sinus depth (M10), and sinus width (M11) in *Q. velutina* also exhibited the same pattern as in *Q. alba* (Table 6). The means of these traits were greater in the outer leaves in both north and south directions. Another interesting pattern was found in the interval between the largest secondary vein and the apex (M3) and maximal lamina width of the left half (M6) where means were greater in only south-facing outer leaves.

Table 6 Morphological traits of south and north facing leaves of *Q. velutina* showing their means and standard error (N = 600).

Traits	South outer	South inner	North outer	North inner
M1	53.63 ± 0.97	48.60 ± 0.86	52.19 ± 1.01	48.33 ± 1.13
M2	44.82 ± 0.88	55.42 ± 1.00	52.21 ± 1.16	59.01 ± 1.19
M3	121.64 ± 1.60	116.12 ± 1.39	120.45 ± 1.59	121.05 ± 1.48
M4	37.27 ± 0.53	37.72 ± 0.57	38.30 ± 0.62	40.11 ± 0.65
M5	89.62 ± 0.98	90.50 ± 1.09	92.59 ± 1.08	96.58 ± 1.33
M6	69.41 ± 0.96	68.84 ± 0.94	71.08 ± 1.04	73.21 ± 1.08
M7	68.87 ± 0.89	69.40 ± 0.88	71.13 ± 0.95	73.15 ± 1.00
M8	32.16 ± 0.71	40.16 ± 0.82	37.16 ± 0.84	41.93 ± 0.90
M9	13.06 ± 0.36	19.49 ± 0.63	17.16 ± 0.55	20.73 ± 0.65
M10	45.82 ± 0.99	35.84 ± 0.93	40.93 ± 1.12	35.33 ± 1.01
M11	27.44 ± 0.55	23.39 ± 0.58	26.16 ± 0.55	25.72 ± 0.53
M12	5071.50 ± 113.55	6110.27 ± 135.68	6017.69 ± 163.09	7133.50 ± 188.45
M13	5100.64 ± 107.50	6364.51 ± 138.55	6026.43 ± 163.77	7176.76 ± 191.68
M14	8178.72 ± 186.67	8352.15 ± 187.50	8803.50 ± 225.87	9405.89 ± 240.71

Table 6 Continued

M15	8158.27 ± 160.60	8510.61 ± 172.23	8707.93 ± 212.08	9463.76 ± 235.09
TLA	10172.14 ± 213.72	12474.78 ± 264.13	12044.12 ± 316.46	14310.27 ± 365.22
LBL	166.46 ± 1.58	171.54 ± 1.77	172.65 ± 2.01	180.06 ± 2.01
TLL	220.09 ±2.25	220.13 ± 2.08	224.84 ± 2.60	228.39 ± 2.46

Sources of FA Variation

A nested ANOVA with general linear model was used to investigate how tree, branch position, leaf position nested within branch position, and all the measured traits influenced variation in FA (Table 7). The final models for each of the 3 species revealed tree, length of the largest secondary vein (M5), and area of leaf blade of the left half (M12) as sources of FA_{area} variation in all the species ($p < 0.05$; N = 600 leaves per species). Position was found to have contributed significantly to FA_{area} differences in *Q. alba* and *Q. velutina* but did not have any significant effect on *Q. palustris*. The nested effect of leaf position within branch position on FA_{area} was not significant for any of the studied species. The effect of outer leaves vs. inner leaves in both north and south directions was significant but the effect of south outer leaves vs. north outer leaves or south inner leaves vs. north inner leaves was not significantly different in *Q. alba*. The effect of position on FA_{area} was marginally significant in *Q. velutina* (Table 7), resulting in significant difference between pairs of means in only south outer vs. north outer. Among the species, petiole length predicted the extent of FA_{area} variation in only *Q. velutina*. All the parameters of the final models to predict the sources of FA_{area} variation in each of the species were significant except position in *Q. palustris* and leaf position nested within branch position in all the species (Table 7). These models had residual plots with no discernible patterns therefore, were eventually accepted (See Appendix Figures 21 for *Q. alba*, 25 for *Q. palustris* and 29 for *Q. velutina*).

The final models to predict the sources of FA_{width} showed that tree influenced FA in the leaf width of *Q. palustris* ($p = 0.033$; N = 600) and *Q. velutina* ($p = 0.011$; N = 600) but not *Q. alba* (Table 7). Position also accounted for FA_{width} variation in both *Q. alba* and *Q. velutina*. Like FA_{area}, position could not explain variation in FA_{width} in *Q. palustris* ($p > 0.05$; N = 600). Contribution of leaf to FA_{width} variation was significant ($p = 0.034$; N = 600) in only *Q. alba* indicating high leaf variability within tree canopy of this species. The effect of M5 on FA_{width} was pronounced in all the 3 species. Other leaf traits which influenced FA_{width} include interval between the largest vein and the apex (M3) and interval between 2 large secondary veins (M4) for *Q. velutina*; interval between the leaf base and the largest secondary vein (M2) and M12 for *Q. palustris* and maximal lamina width of the right half for both *Q. palustris* and *Q. velutina*. All the parameters of the final models to predict the sources of FA_{width} variation in each of the species were significant except tree in *Q. alba*, position in *Q. palustris* and leaf nested within position in *Q. palustris*, and *Q. velutina* (Table 7). The residual plots of these models showed no discernible patterns in the data and were consequently accepted (Figures 4, 5 and 6).

Table 7 Sources of variation for FA, leaf area, shape and lobulation in *Q. alba, Q. palustris* and *Q. velutina* obtained from nested ANOVA showing their *p*-values (*p* = 0.05; N = 600 leaves per species).The *p*-values for traits which did not have significant effect were excluded. T = tree; P = branch position and L = leaf position nested within branch position (See Appendix Tables 22-36 for details)

Trait Sources of variation .

Species	T	P	L(P)	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15
FAarea																		
Q. alba	0.047	0.000	0.983				0.031	0.001							0.000			
Q. palustris	0.008	0.603	0.611					0.018							0.000	0.001		
Q. velutina	0.000	0.041	0.219	0.000		0.008						0.011			0.000			
FAwidth																		
Q. alba	0.069	0.015	0.034					0.000		0.000				0.002				
Q. palustris	0.003	0.594	0.645		0.006			0.000	0.005	0.002			0.047					
Q. velutina	0.011	0.004	0.874			0.035	0.032	0.000							0.000			0.002
Leaf area																		
Q. alba	0.000	0.000	0.183					0.000		0.000	0.000	0.002	0.000	0.000	0.000		0.003	0.000
Q. palustris	0.000	0.000	0.165				0.001	0.002	0.000	0.000	0.000			0.000		0.000	0.000	0.000
Q. velutina	0.000	0.000	0.330	0.008	0.000						0.000	0.000	0.000	0.000			0.000	0.000
Leaf shape																		
Q. alba	0.000	0.894	0.157				0.000	0.000					0.000		0.000	0.000	0.000	
Q. palustris	0.000	0.010	0.197	0.023	0.000	0.000		0.000	0.000		0.000							0.000
Q. velutina	0.000	0.000	0.419		0.000	0.000		0.000	0.000						0.000	0.000		
Lobulation																		
Q. alba	0.000	0.001	0.022			0.000			0.041									
Q. palustris	0.000	0.000	0.695	0.000				0.050	0.005				0.000		0.000	0.000	0.000	0.000
Q. velutina	0.000	0.641	0.812	0.000					0.011		0.004		0.000		0.000	0.000	0.000	0.000

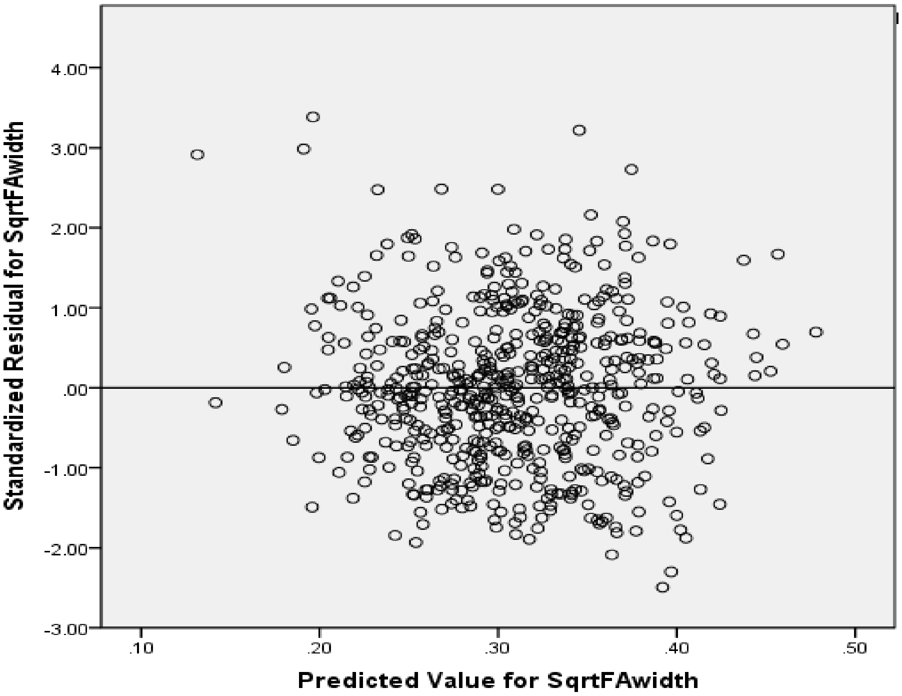

Figure 4 Residual plot of the most fitted model for standardized residual of SqrtFA$_{width}$ vs. predicted residual of SqrtFA$_{width}$ of *Q. alba*. This model was accepted because it did not show any discernible pattern.

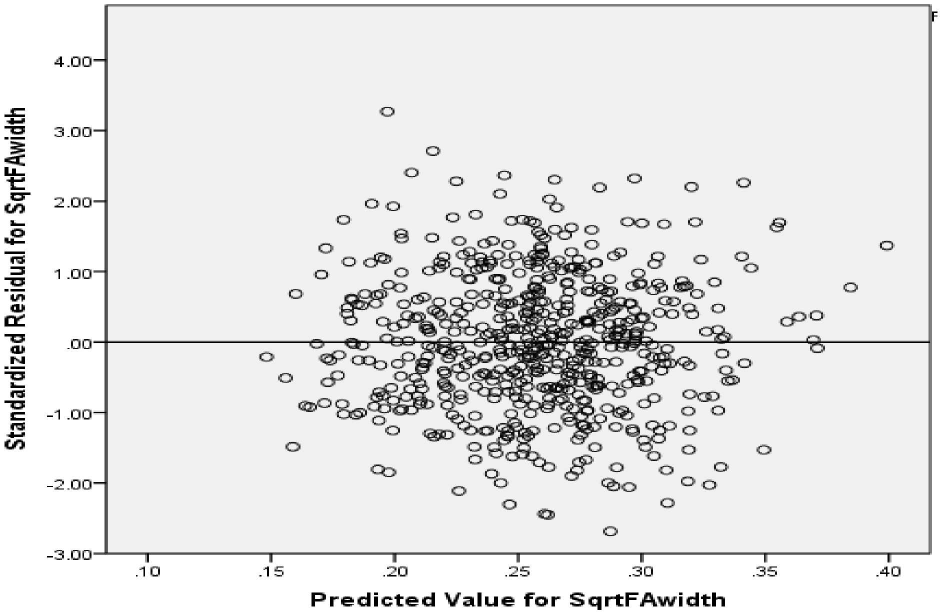

Figure 5 Residual plot of the most fitted model for standardized residual of SqrtFA$_{width}$ vs. predicted residual of SqrtFA$_{width}$ of *Q. palustris*. This model was accepted because it did not show any discernible pattern.

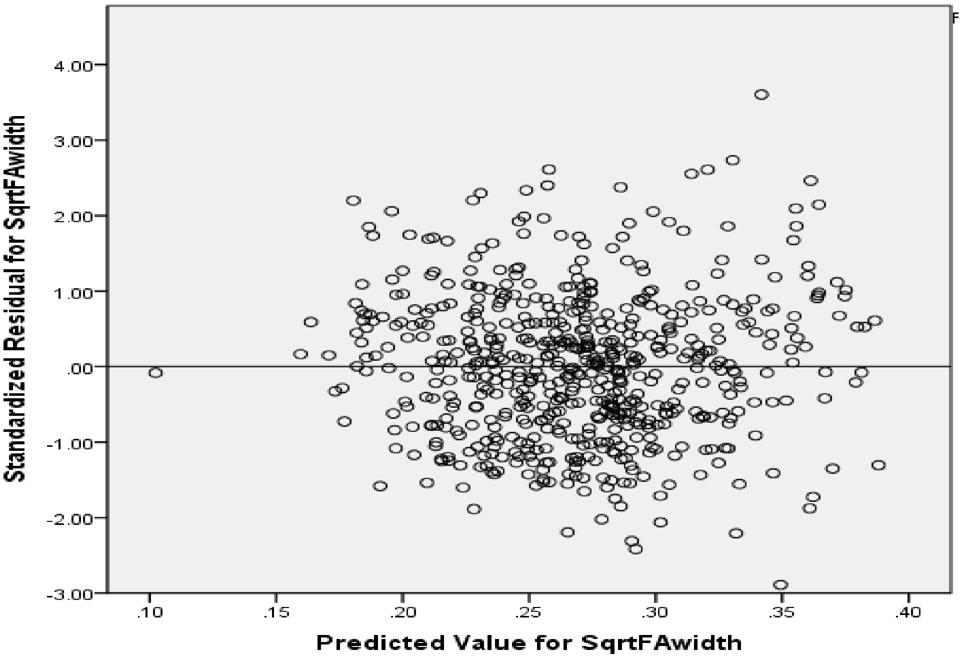

Figure 6 Residual plot of the most fitted model for standardized residual of $\text{SqrtFA}_{\text{width}}$ vs. predicted residual of $\text{SqrtFA}_{\text{width}}$ of *Q. velutina*. This model was accepted because it did not show any discernible pattern.

Sources of Leaf Area Variation

Considering all the variables under investigation, leaf area had the most explanatory variables (Table 7). Both tree and position had high significant effects ($p < 0.001$; N= 600 per species) on leaf area in all the species. However, the effect of leaf position on the individual twigs was not significant in any of the species ($p > 0.05$; N = 600 per species). The effects of interval between basal pairs of sinuses (M8), sinus width (M11), area of leaf envelope (parameter) of the left half (M14), and area of leaf envelope of the right half (M15) on leaf area differences were significant for all the species ($p < 0.001$, N = 600 per species). Unlike FA, the difference in leaf area for *Q. velutina* did not depend on the length of the largest secondary vein. On the other hand, variation in leaf area for *Q. alba* and *Q. palustris* depended on the length of the largest secondary vein. The depth and width of sinus also influenced the variability in leaf area of *Q. alba* and *Q. velutina*. As in FA_{area}, petiole length had a significant effect on leaf area in *Q. velutina*. All the parameters of the final models to predict the sources of leaf area variation in each of the species were significant except leaf position in all the species (Table 7). The residual plots of these models showed no discernible patterns and were consequently accepted (See Appendix Figures 22 for *Q. alba*, 26 for *Q. palustris*, and 30 for *Q. velutina*).

Sources of Leaf Shape Variation

Although the 3 species did not show the same pattern of shape variation, they had common sources of variation (Table 7). Tree, interval between the leaf base and the largest secondary vein (M2), interval between the largest secondary vein and the apex (M3), length of the largest secondary vein (M5), and maximal blade width of the left half (M6) were the main causes of leaf shape variations within *Q. alba*, *Q. palustris,* and *Q. velutina* ($p < 0.001$; N= 600 per species). Position influenced the leaf forms in both *Q. palustris* and *Q. velutina* but not in *Q. alba*. Petiole length was found to be an important source of leaf shape variation in only *Q. palustris* ($p = 0.023$; N = 600) which is an indication that variable petiole lengths have significant influence on leaf shape variability of this particular species. On the other hand, leaf position did not explain shape variation occurring in all the species ($p > 0.05$; N = 600 per species). All the parameters of the final models to predict the sources of leaf shape variation in each of the species were significant except position in *Q. alba* and leaf position in all the species (Table 7). These models had residual plots with no discernible patterns (See Appendix Figures 23 for *Q. alba*, 27 for *Q. palustris*, and 31 for *Q. velutina*).

Sources of Lobulation Variation

The differences in the leaves of *Q. alba* once again contributed to variation in another leaf trait, lobulation- formation of leaf lobes ($p = 0.022$; N= 600). This confirms high leaf variability within different positions of *Q. alba* trees than *Q. palustris* and *Q. velutina* because the effect of leaf on variation was never found significant in these two latter species according to our results (Table 7). The effect of individual trees on lobulation was significant ($p < 0.001$; N= 600 per species) in all the species but the effect of position was significant in only *Q. alba* and *Q. palustris*. The effect of maximal lamina width of the left half (M6) on lobulation was significant in all the species while M1 significant influence on lobulation was observed in *Q. alba* and *Q. palustris*. All the parameters of the final models to predict the sources of leaf lobing variation in each of the species were significant except position in *Q. velutina* and leaf position in *Q. palustris* and *Q. velutina* (Table 7). These models had residual plots with no discernible patterns (Figures 24 for *Q. alba*, 28 for *Q. palustris* and 32 for *Q. velutina*).

Variation in FA Estimated Marginal Means (± standard error) Within Branch Positions of *Q. alba*, *Q. palustris* and *Q. velutina* Predicted by the Final Models

The estimated marginal means of FA_{area} predicted by the final models varied across tree positions and species (See Appendix Table 10). The maximum mean FA_{area} was 0.12 ± 0.012 (mean ± SE) which occurred in the north inner leaves of *Q. alba* while the minimum mean FA_{area} (0.072 ± 0.012) occurred in both south outer and north outer leaves of *Q. palustris*. All the species showed a common trend for FA_{area} across branch positions where FA_{area} means for inner leaves were greater than those of outer leaves except in *Q. velutina* where FA was greater in the north outer leaves (See Appendix Table 10; Figure 7). The estimated average FA_{area} difference between the outer (sun) leaves and inner (shade) leaves of *Q. alba* was significant (Bonferroni test; $p < 0.05$; N = 600; See Appendix Table 11; Figure 7). *Quercus alba* also showed no significant difference between the means of FA_{area} of south outer leaves vs. north outer leaves and south inner vs. north inner leaves. South outer leaves vs. north outer leaves were the only significant pairs of FA_{area} means in *Q. velutina* (See Appendix Table 12; Figure 7). The pairs of means of FA_{area} for *Q. palustris* were not significant (Figure 7) meaning the FA_{area} in this species did not vary among positions within the trees (Table 7).

The highest FA_{width} mean (0.110 ± 0.011) occurred in the north inner leaves of *Q. alba* whereas the least one (0.054 ± 0.011) occurred in the south outer leaves of *Q. velutina* (See Appendix Table 10; Figure 8). The FA_{width} means of the species were greater in the inner leaves except in *Q. palustris* which had greater FA_{width} in the south outer leaves. In *Q. alba*, north outer leaves vs. north inner were the only pairs of means which effects on FA_{area} differed significantly (See Appendix Table 13; Figure 8). In *Q. velutina*, south outer leaves vs. north outer leaves and south outer leaves vs. north inner leaves were the only pairs of means which differed significantly in their effects on FA_{width} (Figure 8).

Variation in Leaf Area, Lobing and Shape Estimated Marginal Means (± standard error) Within Branch Positions of *Q. alba*, *Q. palustris*, and *Q. velutina* Predicted by the Final Models

The estimated marginal means of leaf area predicted by the final models were larger in the inner leaves than those of the outer leaves in all the 3 species ranging from 4395.4 ± $0.001mm^2$ in *Q. palustris* to 12106 ± $0.002mm^2$ in *Q. velutina* (See Appendix Table 10). Interestingly, the south outer and north outer leaves of *Q. alba* had the same average of leaf area (5767.7 ± $0.001mm^2$). Similar observation was also made in both south and north outer leaves of *Q. palustris* which had the same average of leaf area (4395.4 ± $0.001mm^2$). All the pairs of means of leaf area were significant among the positions except south outer leaves vs. north outer leaves and south inner leaves vs. north inner leaves for *Q. alba* and *Q. palustris* (See Appendix Tables 15 and 16 respectively; Figure 9). For *Q. velutina*, all the pairs of means were significant except south inner leaves vs. north inner leaves (See Appendix Table 17; Figure 9).

The estimated marginal means of leaf lobing varied within the trees across the species based on light exposure (See Appendix Table 10). *Quercus velutina* had the highest lobing (4964.9 ± 0.209mm^2) while *Q. alba* had the least lobing (2081.1 ± 0.284mm^2). The inner leaves of both *Q. palustris* and *Q. velutina* exhibited higher lobing than the outer leaves. In contrast, the exposed leaves of *Q. alba* showed higher lobing than the inner leaves (Figure 10). The pairs of estimated marginal means of leaf lobing for *Q. alba* was significant in only north outer vs. north inner leaves ($p < 0.001$; N = 600; See Appendix Table 18; Figure 10). For *Q. palustris*, the pairs of estimated marginal means was significant in only the comparison between south outer leaves and the leaves in other positions (See Appendix Table 19). However, the difference between the estimated marginal means was not significant in *Q. velutina* since light exposure did not have significant influence on lobing ($p > 0.05$; N = 600; Table 6).

Leaf shape showed different patterns of estimated marginal means for each of the species (See Appendix Table 10; Figure 11). In *Q. alba*, the means of leaf shape were greater in the inner leaves while in *Q. palustris* the means were lower in the inner leaves. For *Q. velutina*, the south inner and outer leaves had the same shape but the north inner and outer leaves had different shape. *Quercus palustris* exhibited the highest estimated marginal means of leaf shape while *Q. alba* had the least shape (Figure 11). The differences in estimated marginal means of leaf shape at different positions within the trees of *Q. alba* were marginal (See Appendix Table 10) resulting in non-significant effect of light exposure on leaf shape ($p > 0.05$; N = 600; Table 6). For *Q. palustris*, the estimated means varied significantly in only south outer vs. north inner leaves and north outer vs. north inner leaves (See Appendix Table 20; Figure 11), whereas in *Q. velutina* the estimated means were significantly different except for south outer leaves vs. north outer and inner leaves (See Appendix Table 21; Figure 11).

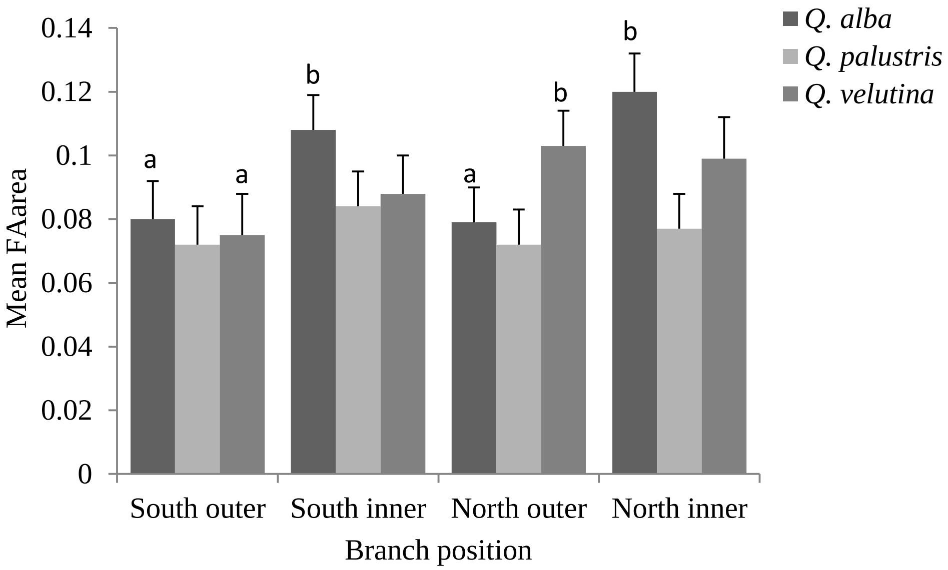

Figure 7 Mean values (± standard error) of FA_{area} for *Q. alba*, *Q. palustris*, and *Q. velutina* in 4 different positions. Different letters for the same species indicate significant difference and the same letters or without letters indicate non-significant difference (Bonferroni test; See Appendix Tables 11and 12).

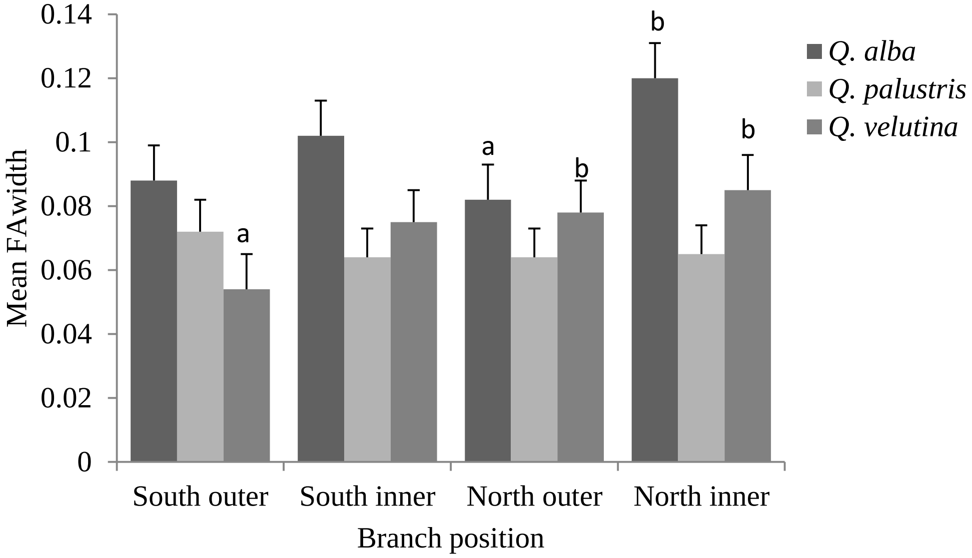

Figure 8 Mean values (± standard error) of FA_{width} for *Q. alba, Q. palustris*, and *Q. velutina* in 4 different positions. Different letters for the same species indicate significant difference and the same letters or without letters indicate non-significant difference (Bonferroni test; See Appendix Tables 13 and 14).

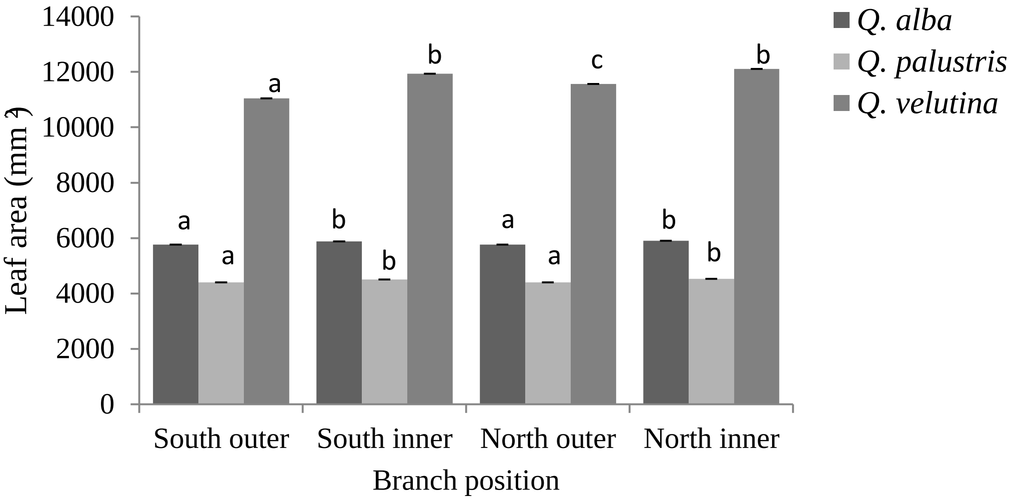

Figure 9 Mean values (± standard error) of leaf area for *Q. alba, Q. palustris*, and *Q. velutina* in 4 different positions. Different letters for the same species indicate significant difference and the same letters or without letters indicate non-significant difference (Bonferroni test; See Appendix Tables 15, 16 and 17).

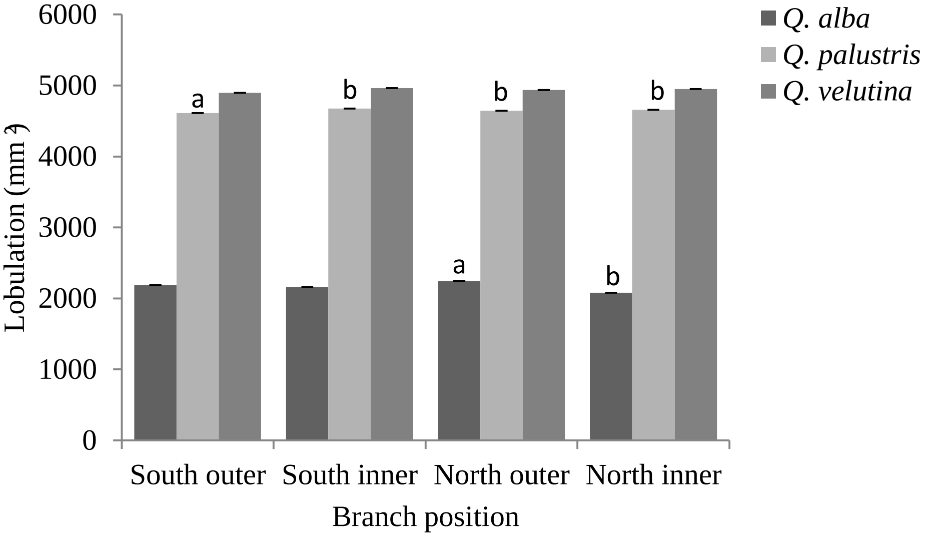

Figure 10 Mean values (± standard error) of lobulation for *Q. alba*, *Q. palustris* and *Q. velutina* in 4 different positions. Different letters for the same species indicate significant difference and the same letters or without letters indicate non-significant difference (Bonferroni test; See Appendix Tables 18 and 19).

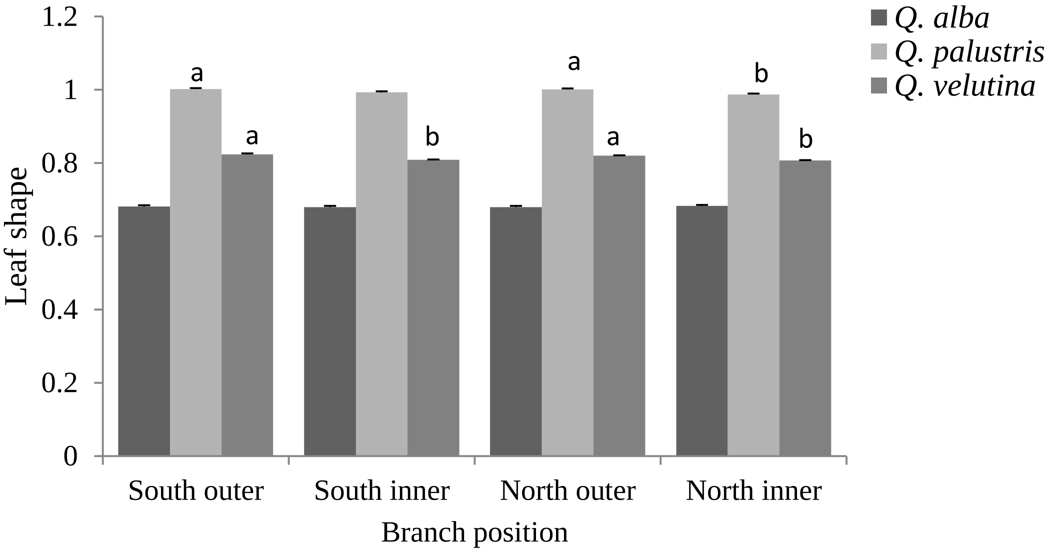

Figure 11 Mean values (± standard error) of leaf shape for *Q. alba*, *Q. palustris* and *Q. velutina* in 4 different positions. Different letters for the same species indicate significant difference and the same letters or without letters indicate non-significant difference (Bonferroni test; See Appendix Tables 20 and 21).

Phenotypic Plasticity

Plasticity index calculated as the absolute difference between the minimum and the maximum mean values was found to be low for all the species. However, the plasticity varied within and among the species (Table 8) which supports our hypothesis that plasticity would vary between the studied species because individual adaptive capacity in response to environmental conditions differs. The species exhibited the same pattern of plasticity in which the sun (north) leaves had greater plasticity than the shade (south) leaves indicating within species variation influenced by light exposure. This supports our hypothesis that sun leaves would show greater plasticity because they experience more severe weather conditions and would employ plasticity to enhance adaptation. *Quercus palustris* exhibited the greatest plasticity, whereas *Q. velutina* had the least plasticity which serves as an evidence of among species plasticity variation as predicted by our hypothesis the species would exhibit variable plasticity

Table 8 Mean phenotypic plasticity index of the morphological traits of *Q. alba, Q. palustris*, and *Q. velutina* in response to light exposure.

	Q. alba		*Q. palustris*		*Q. velutina*	
Traits	South	North	South	North	South	North
M1	0.15	0.16	0.15	0.05	0.09	0.07
M2	0.17	0.12	0.22	0.22	0.19	0.12
M3	0.08	0.04	0.10	0.06	0.05	0.04
M4	0.08	0.04	0.13	0.11	0.01	0.05
M5	0.09	0.05	0.13	0.06	0.01	0.04
M6	0.08	0.05	0.09	0.00	0.01	0.03
M7	0.09	0.06	0.10	0.01	0.01	0.03
M8	0.25	0.24	0.30	0.28	0.20	0.11
M9	0.21	0.22	0.21	0.18	0.33	0.17
M10	0.11	0.14	0.11	0.02	0.22	0.14
M11	0.05	0.13	0.04	0.05	0.15	0.02
M12	0.27	0.22	0.37	0.26	0.17	0.16
M13	0.28	0.24	0.37	0.26	0.20	0.16
M14	0.19	0.12	0.26	0.14	0.02	0.07
M15	0.21	0.13	0.26	0.14	0.04	0.08
TLA	0.26	0.23	0.37	0.26	0.18	0.16
LBL	0.11	0.07	0.14	0.10	0.03	0.04
TLL	0.08	0.04	0.14	0.09	0.00	0.02
Mean value	0.15	0.13	0.19	0.13	0.11	0.08

CHAPTER 4

DISCUSSIONS

Variations in FA Explained by Tree, Branch Position, and Leaf Position

Sources of Variation for FA_{area}

The results of this study revealed that fluctuating asymmetry (FA) varied among *Quercus alba*, *Quercus palustris*, and *Quercus velutina* (Table 7) which does not support our assumption that only random variation occurs within the species. The main effects of individual trees on FA variation in both sides of the leaf area were observed to be marginally significant in *Q. alba* (p = 0.047, N = 30) which is an evidence for our hypothesis that variation in FA would be affected by individual trees. This means that the difference between individual trees of *Q. alba* is less important to cause any significant FA variation in the leaf area which implies that the FA is almost the same among the individual trees. The effect of tree on FA variation observed in this species coincides with the results of Bruschi et al. (2003) who investigated within and among tree variation in leaf morphology of *Quercus petraea* in which trait variation due to individual trees was less than half of the traits measured. Tree observed as low source of FA variation in *Q. alba* in this study may be partly attributed to exposure to similar climatic conditions experienced by the individual trees leading to low expression of phenotypic plasticity because high tree variation occurs among trees experiencing microenvironmental conditions (Gonzalez-Rodriguez and Oyama 2005). Viscosi and Cardini (2011) suggested that space is one factor which contributes to similarity among trees especially those growing close to each other. A careful examination of the sites where these trees were sampled revealed that about 70% of the *Q. alba* trees were sampled from the same location (few meters apart). The growth of these trees in this location may be influenced by the same or similar environmental conditions leading to their subtle individual differences which reflected their marginal effect on FA_{area} variation.

Unlike individual trees, branch position (outer vs. inner) showed highly significant influence on FA_{area} variation in *Q. alba* (Table 7) supporting our hypothesis that the effect of light exposure would be greater on FA variation. This agrees with several previous studies investigating positional effect on leaf morphological traits in *Q. velutina*, *Q. palustris*, and *Q. rubra* (Blue and Jensen 1988); *Q. palustris* and *Q. velutina* (Jensen 1990); *Q. velutina*, *Q. rubra*, and *Q. coccinea* (Ashton and Berlyn 1994); *Q. petraea* (Bruschi et al. 2003) and several temperate deciduous trees (Sack et al. 2006). The obvious implication from these studies is that the outer leaves experience more severe climatic conditions than the inner leaves leading to trait differences. Based on this, some authors hold the view that the important thing to consider in determining the effect of light on leaf morphology within tree canopy is outer vs. inner leaves while cardinal compass direction does not really matter because its contribution to variation is minimal (Baranski 1975; Blue and Jensen 1988). We had a contrary expectation and assumed that the leaves facing southern part of the tree would experience more sunlight exposure than the north-facing leaves, thus giving compass direction a greater influence in terms of leaf morphological variation. However, our results for *Q. alba* clearly showed that the comparison of sunlight effects such as south outer vs. north outer and south inner vs. north inner were not significantly different (See Appendix Table 11). In this situation, compass direction did not affect positions within tree variability in *Q. alba* which agrees with the claim by Baranski (1975) and Blue and Jensen (1988). Leaf position within branch position also had no significant effect on FA in *Q. alba* due to invariable leaves on individual twigs.

For *Q. palustris* the only source of variation was the individual tree: both branch position and leaf position could not explain FA_{area} variation according to our results (Table 7). The non-significant effect of position on FA_{area} variation in *Q. palustris* in our study may be linked to its smaller leaves with wide and deep sinuses which according to Sack et al. (2006) allow more light penetration into tree canopy reducing self-shading. This implies that distribution of sunlight within the tree canopy at different positions may be similar in this species resulting in symmetric leaf area.

The results also showed that *Q. velutina* was sensitive to both individual trees and branch position effects resulting in FA_{area} variation. Ashton and Berlyn (1994) also showed that *Q. velutina* has high plasticity under different light conditions making it more tolerant to dry environment. The ability of this species to tolerate drought may enhance its individual trees to respond differently to microenvironments that may result in variability among individual trees. The records on the sampled trees of this species indicate that they were spatially distributed in their habitats suggesting that they experienced different environmental conditions influencing their variability. The tree variability together with plasticity might have contributed to their significant effect on FA. The prominent effect of branch position on FA agrees with Jensen (1990) results which found branch position as one of the sources of within tree variation in *Q. velutina*. Position is very influential in leaf traits of *Q. velutina* as the leaves on lower branches are typically broader than those on the top branches (Mercker et al. 2006). *Quercus velutina* is noted to be light demanding species (Ashton and Berlyn 1994) so the larger leaves at the reduced irradiance position evolved to maximize light capture for photosynthesis (Rozendaal et al. 2006).

The nested effect of leaf position of individual twigs within branch position on FA_{area} showed the same pattern in all the species (Table 7). The leaf position- differences between individual leaves at a given inner or outer position -could not explain the source of FA_{area} variation in any of the species. This may be because the leaves were collected from the same branch at every position within the tree. Because the leaves from the same tree and branch grow under the control of the same gene and environmental factors, they are usually more similar and any possible variation would be very low (Viscosi and Cardini 2011).

Sources of Variation for FA_{width}

Leaf width is one of the traits which exhibit asymmetry and it is usually used to measure the presence of FA (Hodar 2002; Puerta-Pinero et al. 2008). The final mixed general linear model (GLM) developed to determine the sources of FA_{width} variation in *Q. alba* depicted one of the most interesting results in this study (See Appendix Table 23). This is the only model among the several models we developed for FA analysis that identified leaf position within branch position as a reliable source of FA variation confirming our hypothesis that the effect of leaf position on FA variability would be significant. This result contradicts the findings of Baranski (1975) in which leaf position had less significant effect on *Q. alba* (Blue and Jensen 1988). Considering our experimental design, it was not common to find significant effect of leaf position on FA variation because the leaves were not randomly selected; rather they were taken from the same twig of a branch from every position. Therefore, they are likely to have the same morphology based on the fact that their development may be influenced by the same growth factors. The results of this study have shown that for *Q. alba* various leaf positions on individual twigs at different positions within a tree canopy have significant influence on developmental instability occurring in the leaf width rather than the leaf area. This clearly shows that the traits of the same leaf can respond differently to environmental factors.

The effect of light exposure on developmental instability in leaf width was also examined. The test for effects of positions within the tree revealed that the asymmetric leaf width of *Q. alba* was dependent on the level of light exposure as demonstrated in *Q. ilex* (Hodar 2002). Bonferroni pairwise comparison test (See Appendix Table 13) revealed that north outer vs. north inner were the only positions which effects on FA_{width} differed significantly. This implies that for *Q. alba*, the differences in sun and shade effects on FA_{width} variability were restricted to the north-facing part of the trees indicating the significant effect of cardinal compass direction on within tree variation. It was also observed that the effect of tree on FA_{width} in *Q. alba* was not significant confirming the insensitivity of FA variability to individual trees in *Q. alba*. The asymmetry examined in *Q. alba* of this study confirms a report by Graham et al. (2010) that this species has inconsistent asymmetrical leaves due to unequal number of lobes and sinuses on left and right sides of the leaf blade.

In *Q. palustris*, the pattern of sources for FA_{width} difference was the same as that of FA_{area} variation sources: only the effect of individual trees was significant in addition to a few covariate traits (Table 7). The tree variation occurred because *Q. palustris* is a bottomland dweller (Mercker et al. 2006; Coombes 2010) but sampled trees grow at less moist habitat, thus these individuals modify their leaves to tolerate drought stress as suggested by Dickson and Tomlinson (1996) to increase their adaptive capacities.

The pattern of FA_{width} variation in *Quercus velutina* was similar to its FA_{area}. The 2 sources of FA_{width} variation were tree and position. Bonferroni test showed a significant difference with respect to south outer vs. north outer and south outer vs. north inner positions (See Appendix Table 14). This means that the effect of light exposure on outer vs. outer leaves sampled at opposite locations (south vs. north) within the tree canopy was significantly differently supporting the idea that cardinal compass position influences variation within tree canopy.

Variation in FA Explained by Leaf Covariate Traits

Apart from tree, position, and leaf position, the mixed model general linear model identified other morphological leaf traits (covariates) as sources of FA in all the 3 species (Table 7) which supports our prediction that many of the leaf traits would contribute to FA variation. Each measured trait was found as a source of variation for FA in at least one of the species except 2 traits, interval between basal pairs of sinuses (M8) and area of leaf envelope of the left half of the leaf blade (M14). Among the traits, length of the largest secondary vein (M5) was found to be the most prominent trait which explained FA for both leaf area and width in all the species. This secondary vein emerges from the primary vein and grows toward the leaf margin forming part of the variable venation patterns as a result of flexible self-organized system of the individual species (Scarpella et al. 2006). The significant contribution of this major secondary vein to FA variation may be due to its significant role as structural vein which partly determines the leaf shape and also provides transport and mechanical stability for the leaf (Hong et al. 2005). Another obvious reason may be the correlation between the length of the secondary vein and other leaf traits such as sinus depth and lobe length which was not investigated in this study. Petiole length is known to be an important trait responsible for leaf arrangement to enhance light interception (Bruschi et al. 2000; Gonazalez-Rodriguez and Oyama 2005). Gonazalez-Rodriguez and Oyama (2005) found variable petiole length correlating with other traits influenced by ecological and environmental factors in *Q. affinis* and *Q. laurina*. In view of these reports, we expected that petiole length would account for FA variation in almost all the species. Surprisingly, petiole length accounted for FA variation only in the leaf width of *Q. velutina*. This result is consistent with the characteristic longer petioles of *Q. velutina* (Table 6) which has been identified as a light demanding species (Ashton and Berlyn 1994). The depth and width of sinus did not contribute substantially to FA variation. This may be attributed to the fact that sinus traits are fairly stable within most trees (Blue and Jensen 1988).

Fluctuating Asymmetry and Light Exposure

The results of this study showed that light exposure had significant influence on FA in both *Q. alba* and *Q. velutina* (Table 7). A higher level of FA in response to sunlight exposure was observed in the shade leaves while low level of FA occurred in the sun leaves (Figures 7 and 8). This finding supports our hypothesis that shade leaves would exhibit higher FA because they are less exposed to sunlight and become less adaptive (Hodar 2002; Bruschi et al. 2003). Similar results were also obtained in other studies where plants growing in more adverse environmental conditions were more symmetrical and had low FA levels (Hodar 2002; Cornelissen et al. 2003; Puerta-Pinero et al. 2008). For example, Puerta-Pinero et al. (2008) found higher FA in *Q. pyrenaica* shade leaves than sun leaves for both leaf width and number of leaf lobes and concluded that FA increases with low light radiation. This result agrees with the findings of the current study implying that high developmental instability occurs in leaves which experience low sunlight availability. The FA level decreases in plant organs growing in more light exposure environment because they become more adaptive to the high irradiance over time and their symmetric traits in turn become more symmetrical (Roy & Stanton 1999; Hodar 2002). It appears that light is a factor which stabilizes FA in the outer (sun) leaves. Higher level of FA in shade leaves is an indication of low phenotypic plasticity to cope with low light availability because Graham et al (2010) showed that FA increases in plants with low plasticity to buffer the effects of external environmental conditions. This demonstrates the species inability to compensate their development against limiting light availability (Dongen 2006). However, there are instances where developmental instability has been found to increase in response to more severe environmental conditions (Cowart and Graham 1999; Roy and Stanton 1999; Freeman et al. 2005; Tucic and Miljkovic 2010). For instance, Cowart and Graham (1999) claim that asymmetry varies within *Ficus caricasun*: the sun leaves are more asymmetrical than shade leaves because they experience more adverse environmental conditions such as sunlight, cold, and wind than the shade leaves affecting developmental stability. Evidence from the results of the current study supports the idea that FA decreases with greater light exposure. It also confirms the claim by Tucic and Miljkovic (2010) that the relationship between FA and environmental factors is quite complicated.

Sources of Variation for Leaf Area

The parsimonious GLM showed variable leaf area within individual trees under different light conditions across the species (Table 7). As reported in several studies (Bruschi et al. 2003; Ponton et al. 2004; Sack et al. 2006), the leaf area of the studied species also followed the usual pattern; sun leaves were smaller while shade leaves were larger in size (Figure 9) indicating a greater effect of light exposure on leaf area (Ponton et al. 2004). This leaf area modification in response to light exposure supports our hypothesis that sunlight exposure would have greater effect on leaf morphology.

The effect of tree on leaf area was also significant in all the 3 species. The dependence of leaf area variation on tree shows how individual trees vary within every species. The leaf area variation caused by the individual trees may be due to the particular conditions in which each tree was growing. This may include the effect of shading by campus buildings and other trees. Differences in leaf area have significant ecological implications. For instance, the expansion of a leaf under water stress habitat is constrained by insufficient water delivery within the lamina (Zwieniecki et al. 2004).The smaller size of sun leaves prevents water loss through transpiration and larger shade leaves maximize light harvest for photosynthesis (Rozendaal et al. 2006; Franks and Beerling 2009). According to Rozendaal et al. (2006), leaf area is influenced by sunlight resulting in organ modification to produce small leaves to prevent overheating which is consistent with this study's results. Differences in leaf area may be used for adaptation to various levels of irradiance and other environmental factors. The relationship between leaf area and the environment has ecological importance. Sun leaves are known to be small with high stomatal density and vein density (Brodribb and Jordan 2011). These characteristics have been used in fossil leaves to estimate palaeoclimate (Poole et al. 1996; Zwieniecki et al. 2004). While light exposure accounted for leaf area variation, different leaves on individual twigs within the tree canopy could not explain leaf area variation of any of the species because they were invariable when examined within the positions of the individual trees.

Many traits also accounted for differences in leaf area within the species (Table 7). The explanation of leaf size differences by other leaf traits is an indication of evolution of variation occurring within the individual traits (Dunbar-Co et al. 2009). Petiole length influenced leaf size variation in only *Q. velutina.* Longer petioles have been found to be associated with leaf size (Dunbar-Co et al. 2009) which reflects the leaf size dependence on longer petiole of *Q. velutina* in this study. Sinus traits and leaf parameter were found to be the major sources of leaf size diversity apart from tree and position in all the three species. The results also demonstrated inverse relationship between leaf area and sinus traits- smaller (sun) leaves had deeper and wider sinus in both *Q. alba* and *Q. velutina* but for *Q. palustris* surprisingly, the relationship was the reverse. This may be due to the fact that inner leaves of *Q. palustris* also have deep and wide sinuses like the outer leaves. Another different feature found with *Q. palustris* was the longer petiole length of inner leaves than the outer leaves. This suggests that the reduced self-shading effect experienced by *Q. palustris* trees, decreases competition for light among the outer leaves resulting in the shorter petiole length as compared to the inner leaves.

Sources of Leaf Shape Variation

The results of this study indicate that all the species demonstrated variable shapes within individual trees (Table 7). This means that plants evolve and maintain appropriate shapes that increase their fitness in a given environment (Klingenberg 2010). The effects of individual trees light exposure and leaf covariate traits on shape variation varied across the species. Individual trees explained shape variation in all the species while light exposure accounted for shape variation in only *Q. palustis* and *Q. velutina*. This result is contrary to that of a study by Rozendaal et al. (2006) who did not find much difference between the shapes of sun and shade leaves of 38 tropical tree species and concluded that light does not alter leaf shape.

Quercus palustris and *Q. velutina* exhibited the same patterns of within-tree and between tree shape variability (Table 7). Different types of leaf shapes occurring within these two species are dependent of tree and branch position. The effect of individual tree on leaf shape was highly significant in both species. The shape of sun and shade leaves varied significantly in both *Q. palustris* and *Q. velutina* (Figure 11). Although Jensen (1990) found the same sources accounting for shape variation in these two species, they did not show the same patterns of variation. He found that top (sun) and lower (shade) leaves within tree canopy of *Q. palustris* had similar leaf shapes while in *Q. velutina* the shapes of the leaves were different. Again, he found that shape diversity among trees within the species was more pronounced in *Q. velutina* than *Q. palustris*. The disparity between the two results may be due to the difference in sample size. A large sample size (1800 leaves from 90 trees) was used for this study while he used a small sample size by collecting leaves from only 2 adult trees per species.

The pattern of leaf shape difference sources explained by leaf covariate traits was similar for all the species (Table 7). The leaf shape depended on the interval between the leaf base and the largest secondary vein (M2), interval between the largest secondary vein and the apex (M3), length of the largest secondary vein (M5) and maximal blade width of the left half (M6) for all the species. Petiole length (M1), interval between basal sinuses (M8), and leaf envelop of the right half (M15) in addition contributed to leaf form variation in *Q. palustris*. The relationship between leaf shape and these traits is an indication of co-variation needed for integration of plant function (Dunbar-Co et al. 2009). Variation between leaf positions within branch position in the tree did not account for leaf shape variability in any of the species. This may be due to similar sizes of the leaves observed within the positions of the tree canopy of the individual species.

Sources of Lobulation Variation

Leaf lobing varied significantly across the species (Table 7). Three sources: tree, position, and leaf position on the twig accounted for lobe variation in *Q. alba*. Sun leaves of *Q. alba* were more lobed than shade leaves (Figure 10). This agrees with similar results by Sack et al. (2006) which showed lobing increase from inner to outer leaves in *Q. rubra* and *S. albidum* and Valladares et al. (2000) which indicated greater lobes in sun leaves than shade leaves of both *Q. coccifera* and *Q. ilex*. These results imply modification of leaf shape by light exposure (Rozendaal et al. 2006). Greater lobing in the sun leaves allows light penetration into the tree canopy to enhance light capture for photosynthesis (Sack et al. 2006). Leaf position influenced lobing variation in only *Q. alba* which may be attributed to asymmetric nature of the lobes and sinuses usually exhibited by the left and right sides of the leaves (Graham et al. 2010).

Tree and position accounted for lobing differences in *Q. palustris* (Table 7). Contrary to *Q. alba*, lobing increased from outer to inner leaves for both *Q. palustris* and *Q. velutina* (Figure 10) similar to *A. saccharum*, *B. alleghaniensis*, and *L. tulipifera* reported by Sack et al. (2006). However, the difference was not significant with respect to branch position for *Q. velutina*. The results also revealed lobing dependence on leaf size across the species in different patterns (See Appendix Table 10; Figure 10). For *Q. alba*, lobing had an inverse relationship with leaf size while direct relationship between the 2 traits occurred in *Q. palustris* and *Q. velutina*.

Effects of Tree and Leaf Position on Leaf Morphology, and FA

The results of this study showed that individual trees had greater effects on leaf morphology and FA variability across the species (Table 7). The FA analyses for all the species indicated tree effects on FA variation in all the species except *Q. alba* which was marginally significant. According to Rozendaal et al. (2006) plasticity is greater in tall trees reducing the level of FA (Graham et al. 2010). This implies that tree height may have a link with FA because increasing height reduces shade from neighboring trees (Niklas 1995) which in turn affects FA level as our results showed FA increase in shade leaves (Figures 7 and 8). We also recognized that trees of the species sampled for this study were quite tall and aged on average (Table 1). Hence, we suspected tree height and diameter as possible factors that may account for significant effects of individual trees on FA variability. However, our investigation indicated that the estimated height and diameter of the tree have no influence on FA variation in *Q. alba*, *Q. palustris*, and *Q. velutina* (Tables 37-41).

Because these trees were sampled from a university campus where there are many tall buildings and other trees, it is possible for individual trees to experience microclimatic conditions within the campus. This suggests that the shading effect of the tall buildings and neighboring trees may be partly responsible for the greater effects of the individual trees on variation within the species. The presence of tall buildings may explain why tree height did not account for FA variation because their effect may be interfered by shade from the buildings. In addition, genetic variation among the trees within the species was suspected to be partly responsible for tree contribution to FA variation because low FA is an indication of higher genetic variation for adaptation to unfavorable environmental conditions (Graham et al. 2010).

Leaf position on individual twigs had less significant effect on leaf morphology and FA variations (Table 7). This result agrees with that of Baranski (1975) according to Blue and Jensen (1988) in which leaf location on a twig was not found to be a significant source of variation within tree.

Phenotypic Plasticity and Light Exposure

Phenotypic plasticity varied within individual trees and among species in response to sunlight exposure (Table 8). This indicates individual species adaptive capacities to cope with several unfavorable climatic conditions as found in *Q. petraea* (Bruschi et al. 2003). Although plasticity level varied among the species, the patterns of variation within the tree canopy were similar. Higher plasticity occurred in the exposed leaves which is consistent with their low FA while low plasticity found in the shade leaves is also consistent with their higher FA level (Figures 7 and 8) which supports a claim by Graham et al. (2010) that increase in plasticity causes low FA level. Greater plasticity in the sun leaves confirms the species plastic response to greater light availability and it is also associated with light exploitation and resource acquisition (Gratani 2006). The greatest plasticity observed in *Quercus palustris* (wet tolerant species) and the least plasticity observed in *Q. velutina* (dry tolerant species) agrees with the study of Markesteijn et al. (2007) in which wet forest species had greater plasticity and dry forest species had lower plasticity in response to light. This suggests that *Q. palustris* had higher leaf responsiveness to light whereas *Q. velutina* had low leaf responsiveness to light (Valladares et al. 2000). The greater plasticity demonstrated by *Q. palustris* may be responsible for its ability to grow in wide range of soils resulting in being the most widely distributed planted oak in North America (Coombes 2010). On the other hand, *Q. velutina* is a light demanding species (Ashton and Berlyn 1994) and light was not a limiting factor in the habitat where the species were sampled so high plasticity for light was not necessary for resource acquisition (Markesteijn et al. 2007). This study also showed that *Q. palustris* demonstrated variation in leaf area, shape and lobulation but not FA in response to light (Table 7). This means that FA in *Q. palustris* is not sensitive to light indicating low developmental instability. This suggests that there are other mechanisms which buffer its development against the effects of varying climatic conditions. In view of this, high plasticity exhibited by *Q. palustris* is an evidence of the important role of plasticity in adaptation to cope with various environmental effects (Valladares et al. 2000; Graham et al. 2010). The intermediate plasticity exhibited by *Q. alba* could be because this species has been found to be more tolerant to shade than *Q. velutina* (Phelps et al. 1976) and increase in plasticity is necessary for adaptation to available greater light exposure.

Leaf traits related to leaf area had the greatest plasticity in both *Q. alba* and *Q. palustris*, while the interval between the apical sinuses had the greatest plasticity in *Q. velutina* (Table 8). The greater plasticity in leaf area exhibited by *Q. alba* and *Q. palustris* suggests leaf modification to maximize resource acquisition in their shade leaves and prevention of water loss in the sun leaves (Franks and Beerling 2009). Although our study indicated low plasticity occurring in *Q. alba*, *Q. palustris*, and *Q. velutina*, it has been confirmed that these species are capable of producing variable phenotypes to cope with the present and future climatic changes.

Characteristics of *Q. alba*, *Q. palustris* and *Q. velutina*

Generally *Q. alba*, *Q. palustris*, and *Q. velutina* are deciduous species with variable leaf morphology. These species have simple leaves but differ in size, shape, and blade margins (Jensen 1990). *Quercus alba* has short petiole, 7 to 9 narrow lobes with deep sinuses and round margins. The leaf shape is obovate to elliptic and size is about 20 x 8cm. This species is widely distributed in China, Taiwan, Korea, and Japan growing mainly in the mountains (Mercker et al. 2006; Coombes 2010). *Quercus palustris* has long petiole, 5 to 7 lobes at right angle to the midvein having pointed margins, wide and deep sinuses. The leaf shape is elliptic to oblong in outline and has leaf size of about 15 x 20cm. it is commonly found in the southern Canada and Eastern United State growing preferably in moist and poorly drained soils but thrives well in wide range of soils (Mercker et al. 2006; Coombes 2010). *Quercus velutina* has longer petioles, 5 to 9 lobes with wide and deep sinuses. The leaf shape is elliptic to oblong or obovate and leaf size of about 10 x 5cm. This species is widely distributed and commonly found in southern United State growing in moist woodlands or riverbanks (Mercker et al. 2006; Coombes 2010). Results of this study show that *Q. velutina* have leaves with widest and deepest sinuses in outer leaves while *Q. alba* have leaves with narrowest and shallowest sinus dimensions (Tables 4, 5, and 6). *Quercus alba* and *Q. velut*ina showed a common pattern where the sinus traits were greater in the sun leaves demonstrating higher leaf responsiveness to light exposure. In contrast, the sinus traits were greater in the shade leaves for *Q. palustris* indicating more leaf responsiveness to shade effect.

It is well known that these species grow in wide range of habitats with different climatic conditions influencing their growth and development (Dickson and Tomlinson 1996). These species also respond to climatic conditions differently which can be interpreted as different adaptability to changes in the environment (Dickson and Tomlinson 1996). For example, Ashton and Berlyn (1994) found that *Q. velutina* is highly tolerant to light which may enhance water use efficiency (Dickson and Tomlinson 1996). Although this study's results indicate *Q. velutina* response to light exposure, it was not found to be the most light tolerant species (Table 8). The greatest plasticity exhibited by *Q. palustris* may be interpreted as the reason why it is the most dominant oak species planted at East Tennessee State University Arboretum (Faa et al. 2012).

The results of this study (Figures 7 and 8) support the idea that most *Quercus* species have asymmetric leaves (Borazan and Babac 2003). Among our studied species, *Q. alba* exhibited the greatest FA indicating the occurrence of high developmental instability. *Quercus palustris* demonstrated the least FA while *Q. velutina* showed intermediate FA. It can be suggested that *Q. palustris* is more capable of compensating its development against the effects of environmental conditions such as light resulting in low measure of developmental instability (Graham et al. 2010). This implies that *Q. palustris* may have the most stabilized leaf development.

CHAPTER 5

CONCLUSIONS

This study revealed that leaf morphology, plasticity, and fluctuating asymmetry (FA) vary within the tree canopy of *Q. alba, Q. palustris,* and *Q. velutina* in response to light exposure which had greater effects on the species. Fluctuating asymmetry was greater in the inner (shade) leaves which supports our hypothesis that shade leaves would exhibit greater FA because they experience less severe environmental conditions, thus, become less plastic (Hodar 2002; Bruschi et al. 2003). This implies that developmental instability is greater in the shade leaves of these species. Again, low FA demonstrated by the outer (sun) leaves is contrary to similar studies in other species *Ficus caricasun* (Cowart and Graham 1999); *Cnidoscolus stimulosus* and *Ipomoea pandurata* (Freeman et al. 2005) and *Iris pumila* (Tucic and Miljkovic 2010) where FA increase was found in leaf responsiveness to unfavorable environmental conditions. Plasticity was found to be greater in outer (sun) leaves of all the studied species which is an indication of the species responsiveness to light exposure (Graham et al. 2010). *Quercus palustris* exhibited the greatest phenotypic plasticity which may enhance adaptation to varying climatic conditions that may contribute to its wide spread at East Tennessee State University Arboretum as the most dominant species of the planted oaks (Faa et al. 2012). This may also be due to its ability to grow in different soil types and the ease of transplanting (Coombes 2010). Leaves which showed greater plasticity had low FA and vice versa suggesting an inverse relationship between the 2 mechanisms. Tree also had significant effects on leaf morphology and FA which may be attributed to shading effects from tall buildings and other trees. Other factors such as genetic variations among the trees are also suspected to be possible causes of tree effects because they can enhance adaptation to climate change (Graham et al. 2010). In addition, this study revealed several leaf covariate traits as sources of leaf morphological and FA variations which is an indication of co-variation needed for integration of plant function (Dunbar-Co et al. 2009). Although the experimental design was complex, it showed that cardinal compass direction has significant influence on variation within tree canopy.

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APPENDICES

APPENDIX A

Test for Antisymmetry in *Q. alba*, *Q. palustris*, and *Q. velutina*

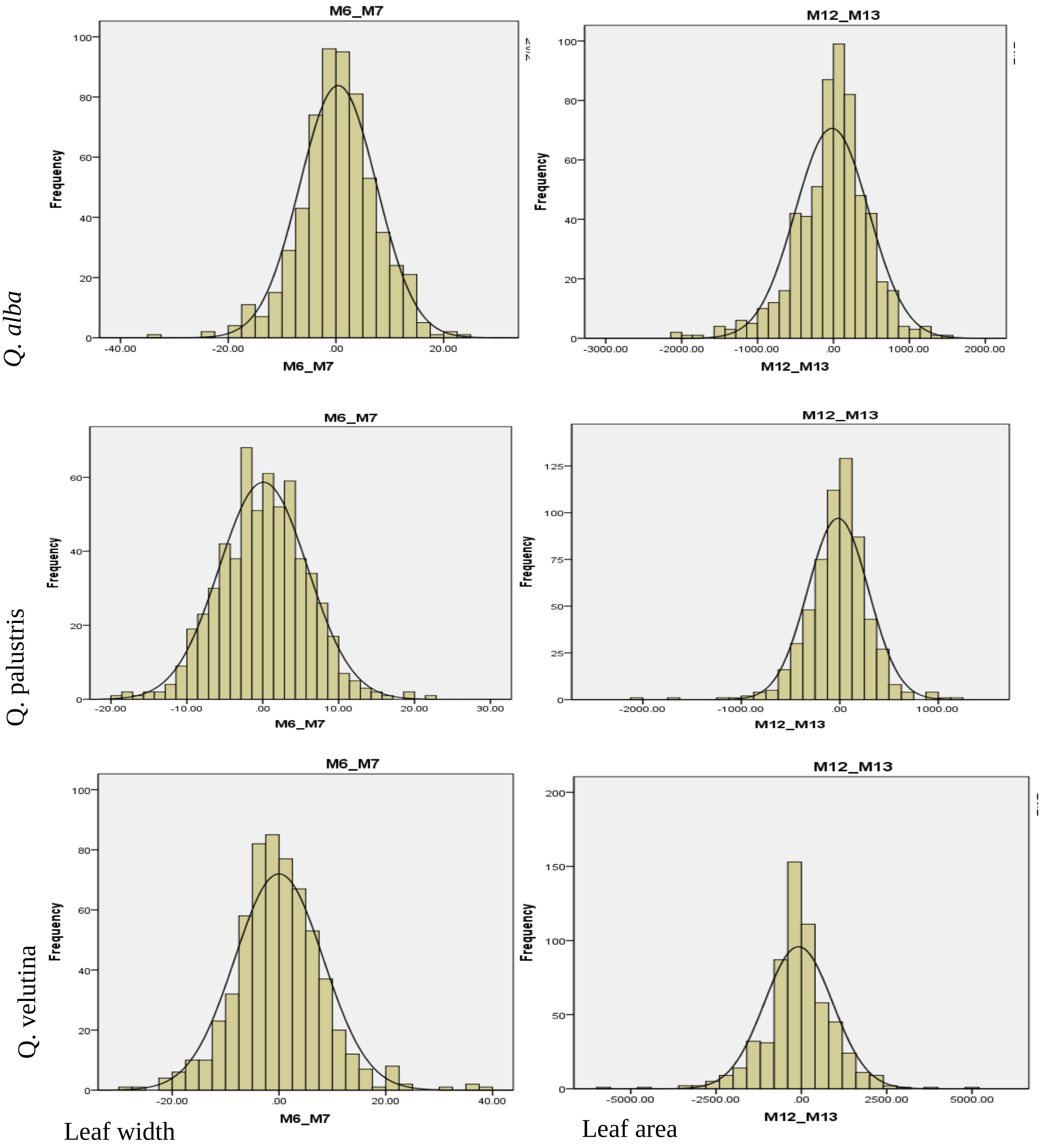


Figure 12 Signed FA for leaf width and area to check antisymmetry. None of the histograms showed bimodal distribution about the mean indicating the absence of antisymmetry.

APPENDIX B

Normality Tests for Data Distribution

Table 9 Normal distribution tests for measured traits using Kolmogorov Smirnov for *Q. alba*, *Q. palustris and Q. velutina*. Most of the traits met normal distribution assumption ($p > 0.05$) and those which did not meet the assumption ($p < 0.05$) were retained because their histograms showed normal distribution.

	Q. alba		*Q. palustris*		*Q. velutina*	
Trait	Kolmogorov Smirnov	*p*	Kolmogorov Smirnov	*p*	Kolmogorov Smirnov	*p*
M1	0.050	0.001	0.042	0.015	0.031	0.200
M2	0.047	0.003	0.026	0.200	0.049	0.200
M3	0.047	0.003	0.032	0.192	0.033	0.190
M4	0.037	0.053	0.038	0.040	0.026	0.200
M5	0.035	0.077	0.035	0.071	0.038	0.038
M6	0.032	0.191	0.034	0.099	0.037	0.050
M7	0.047	0.003	0.022	0.200	0.034	0.089
M8	0.053	0.000	0.042	0.013	0.040	0.021
M9	0.022	0.200	0.034	0.093	0.031	0.200
M10	0.026	0.200	0.025	0.200	0.025	0.200
M11	0.070	0.000	0.036	0.060	0.042	0.016
M12	0.032	0.200	0.015	0.200	0.019	0.200
M13	0.032	0.200	0.022	0.200	0.022	0.200
M14	0.034	0.098	0.039	0.032	0.026	0.200
M15	0.021	0.200	0.034	0.095	0.027	0.200
TLA	0.022	0.200	0.020	0.200	0.021	0.200
TLE	0.025	0.200	0.038	0.042	0.029	0.200
LBL	0.027	0.200	0.032	0.199	0.020	0.200
TLL	0.021	0.200	0.026	0.200	0.029	0.200

APPENDIX C

Boxplots for Measured Traits of *Q. alba*, *Q. palustris*, and *Q. velutina*

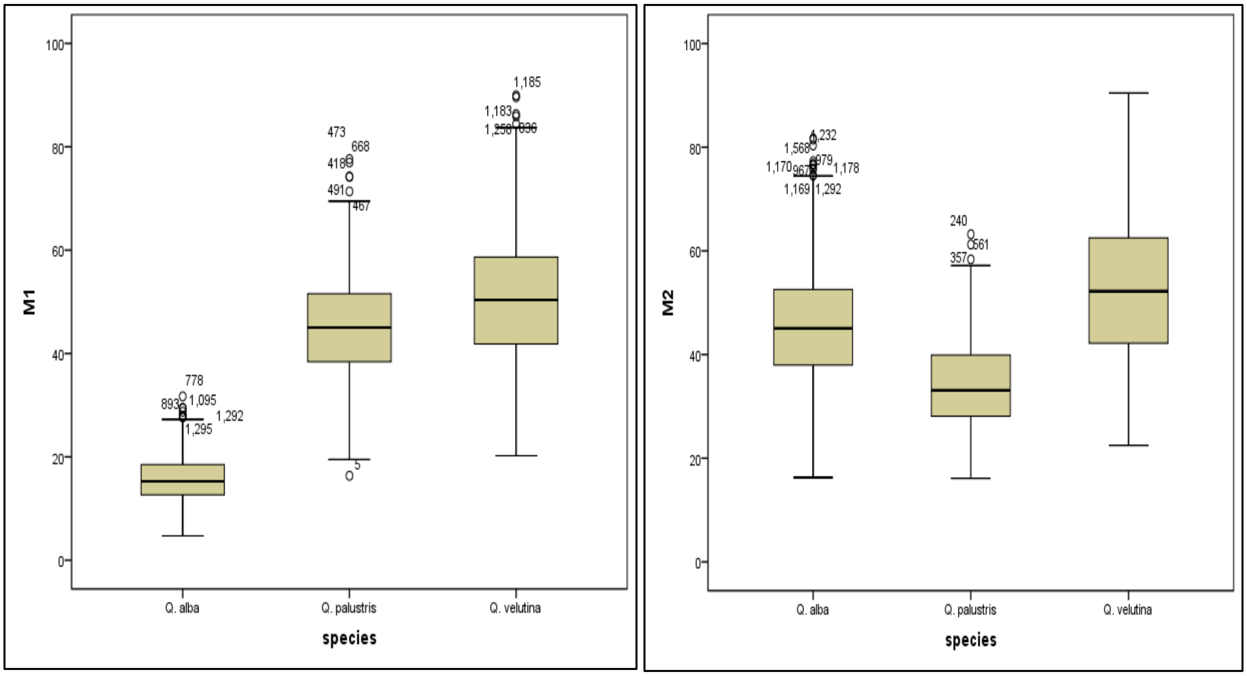


Figure 13 Boxplot of petiole length (M1) (left) and interval between the leaf base and the largest secondary vein (M2) (right) of *Q. alba*, *Q. palusrtis*, and *Q. velutina* showing outliers in the data.

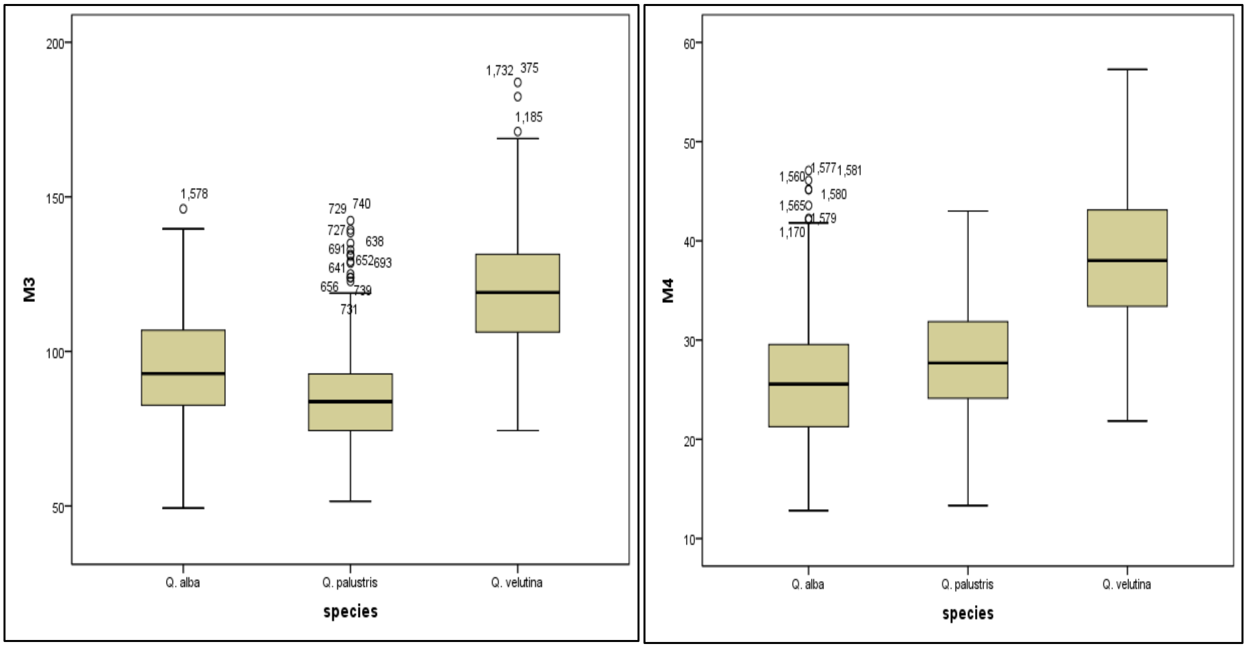


Figure 14 Boxplot of interval between the largest secondary vein and the apex (M3) (left) and interval between two large secondary veins (M4) (right) of *Q. alba*, *Q. palusrtis*, and *Q. velutina* showing outliers in the data.

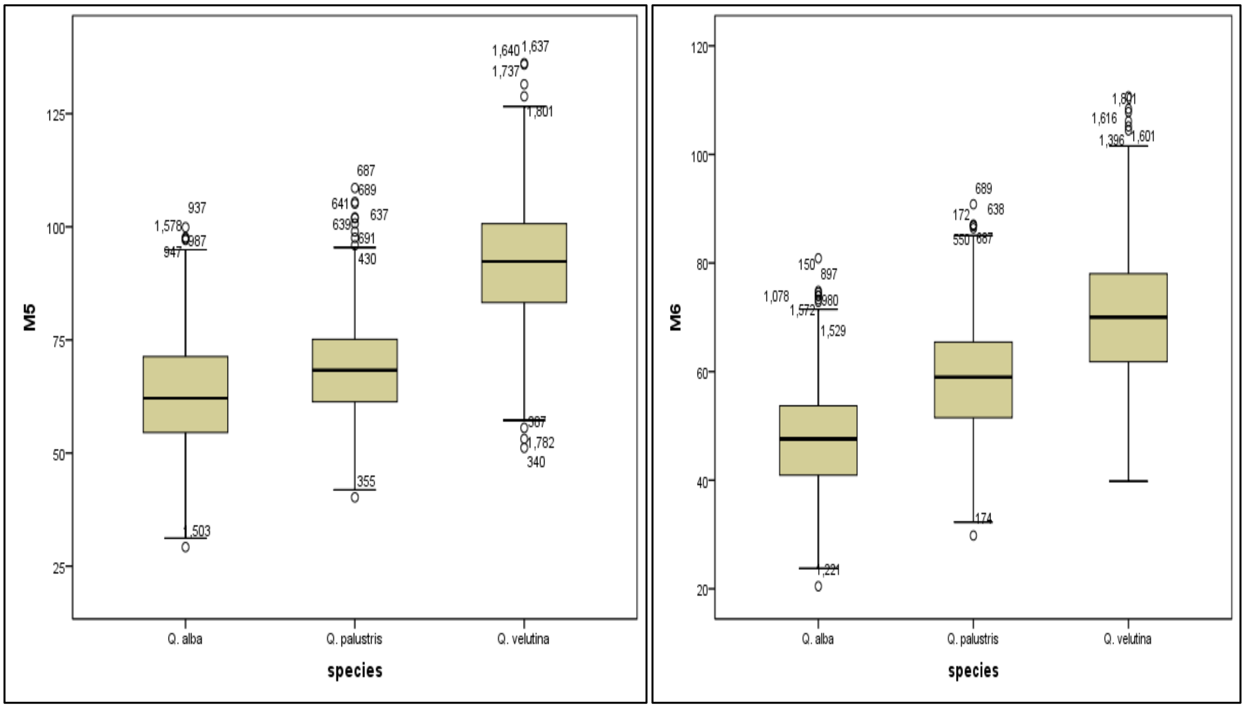


Figure 15 Boxplot of length of the largest secondary vein (M5) (left) and maximal blade width of the left half (M6) (right) of *Q. alba*, *Q. palusrtis*, and *Q. velutina* showing outliers in the data.

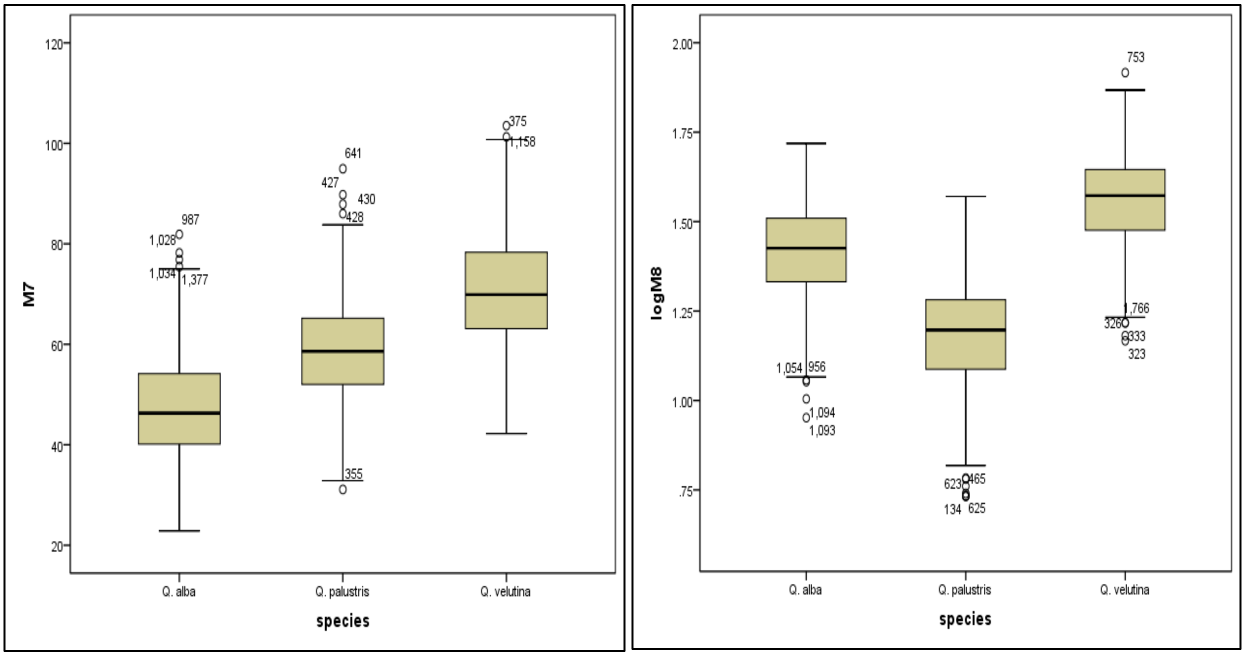


Figure 16 Boxplot of maximal blade width of the right half (M7) (left) and interval between basal pairs of sinuses (M8) (right) of *Q. alba*, *Q. palusrtis*, and *Q. velutina* showing outliers in the data.

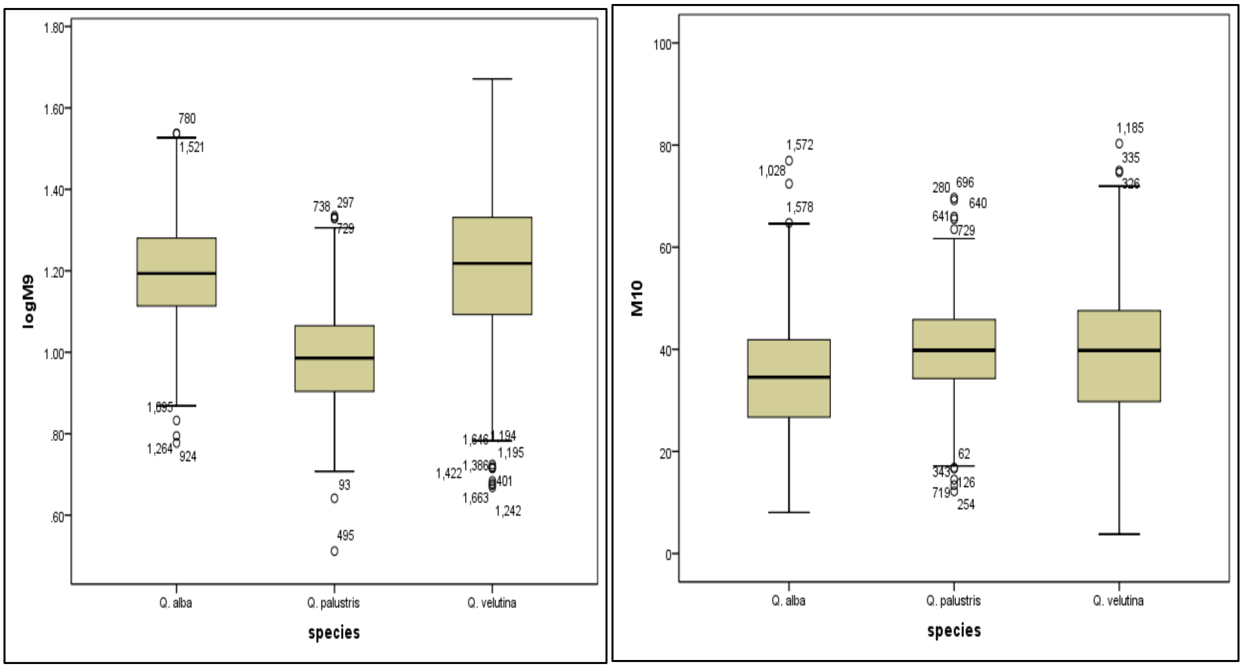


Figure 17 Boxplot of interval between apical pairs of sinuses (M9) (left) and Sinus depth next to M5 (M10) (right) of *Q. alba*, *Q. palusrtis*, and *Q. velutina* showing outliers in the data.

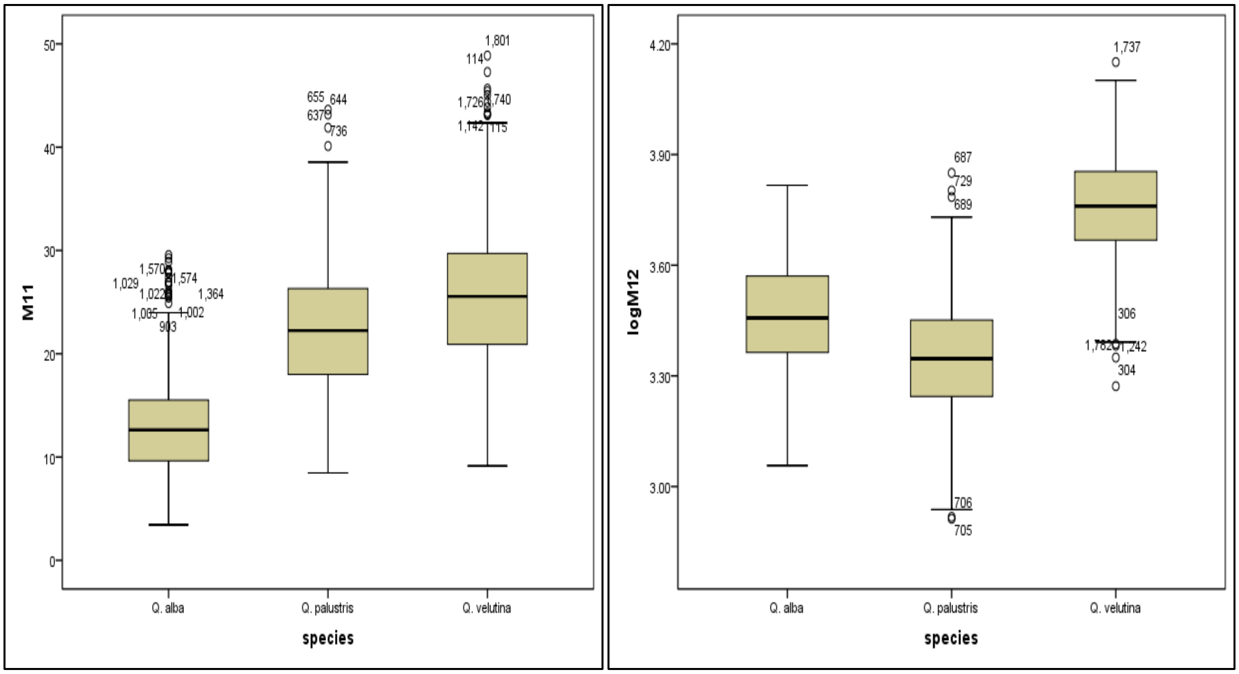


Figure 18 Boxplot of sinus width (M11) (left) and area of the left half of the leaf blade (M12) (right) of *Q. alba*, *Q. palusrtis*, and *Q. velutina* showing outliers in the data.

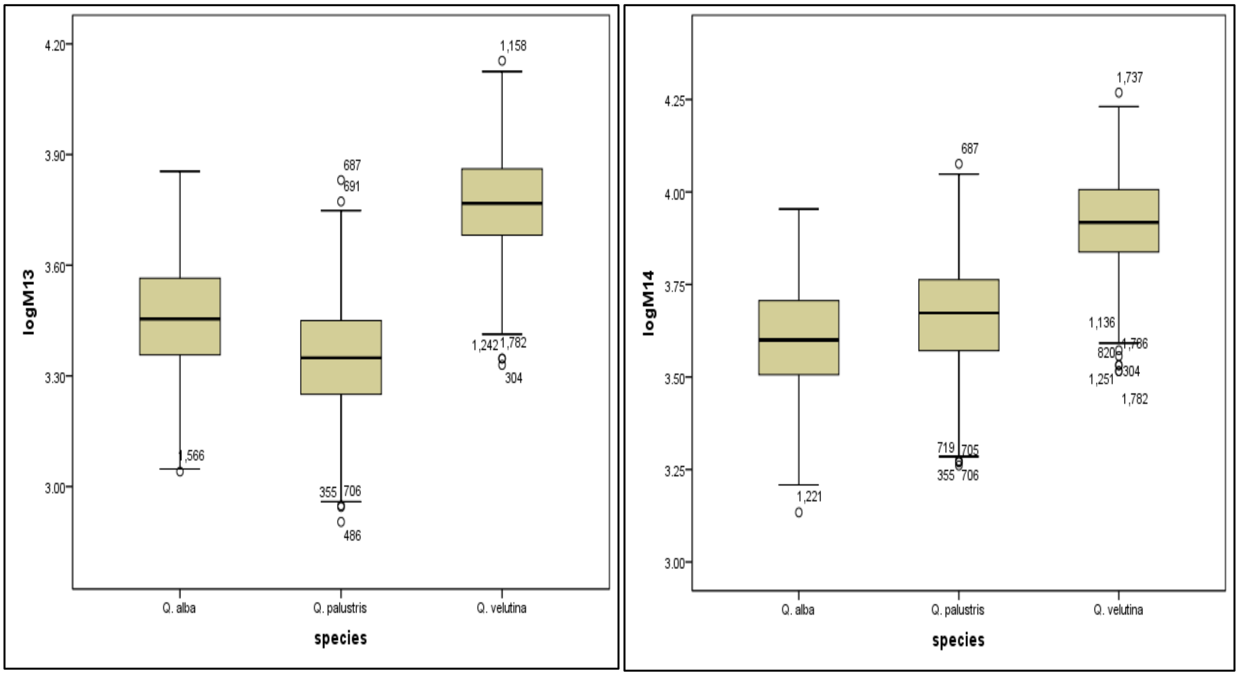


Figure 19 Boxplot of area of the right half of the leaf blade (M13) (left) and area of leaf envelope (parameter) of the left half of the leaf blade (M14) (right) of *Q. alba*, *Q. palusrtis*, and *Q. velutina* showing outliers in the data.

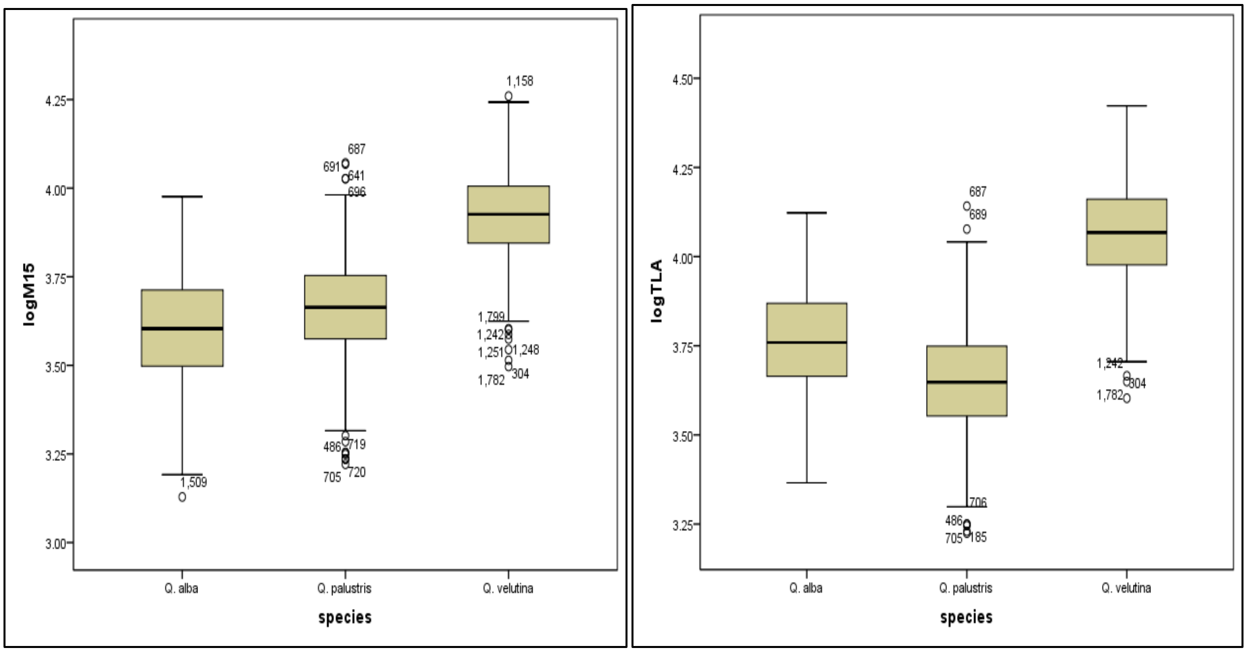


Figure 20 Boxplot of area of leaf envelope of the right half of the leaf blade (M15) (left) and LogTLA (leaf area) (right) of *Q. alba*, *Q. palusrtis*, and *Q. velutina* showing outliers in the data.

APPENDIX D

Estimated Marginal Means and Standard Error of the Traits of *Q. alba*, *Q. palustris*, *and Q. velutina*

Table 10 Estimated marginal means and standard error of dependent variables at 4 different branch positions for *Q. alba*, *Q. palustris*, and *Q. velutina* predicted by the final models.

Trait	Mean ± SE (mm) for tree position		
Position	*Q. alba*	*Q. palustris*	*Q. velutina*
$\mathrm{FA}_{\mathrm{area}}$			
South outer	0.08 ± 0.012	0.072 ± 0.012	0.075 ± 0.013
South inner	0.108 ± 0.011	0.084 ± 0.011	0.088 ± 0.012
North outer	0.079 ± 0.011	0.072 ± 0.011	0.103 ± 0.011
North inner	0.12 ± 0.012	0.077 ± 0.011	0.099 ± 0.013
$\mathrm{FA}_{\mathrm{width}}$			
South outer	0.088 ± 0.011	0.072 ± 0.010	0.054 ± 0.011
South inner	0.102 ± 0.011	0.064 ± 0.009	0.075 ± 0.010
North outer	0.082 ± 0.011	0.064 ± 0.009	0.078 ± 0.010
North inner	0.12 ± 0.011	0.065 ± 0.009	0.085 ± 0.011
Leaf area (mm^2)			
South outer	5767.7 ± 0.001	4395.4 ± 0.001	11040.8 ± 0.003
South inner	5874.9 ± 0.001	4508.2 ± 0.001	11939.9 ± 0.002
North outer	5767.7 ± 0.001	4395.4 ± 0.001	11561.1 ± 0.002
North inner	5902.1 ± 0.001	4529 ± 0.001	12106 ± 0.002
Shape			
South outer	0.681 ± 0.004	1.002 ± 0.003	0.824 ± 0.002
South inner	0.680 ± 0.003	0.993 ± 0.003	0.809 ± 0.001

Table 10 Continued

North outer	0.680 ± 0.003	1.001 ± 0.003	0.820 ± 0.001
North inner	0.683 ± 0.003	0.987 ± 0.003	0.807 ± 0.001
Lobulation (mm^2)			
South outer	2189.8 ±0.286	4613.2 ± 0.001	4897.6 ±0.259
South inner	2159.6 ±0.260	4677.4 ± 0.001	4964.9 ±0.209
North outer	2240.7 ±0.260	4645.2 ± 0.001	4935.2 ±0.194
North inner	2081.1 ±0.284	4655.9 ± 0.001	4952.4 ±0.231

APPENDIX E

Bonferroni Pairwise Comparison Test for *Q. alba*, *Q. palustris*, and *Q. velutina*

Table 11 Bonferroni pairwise comparisons test to determine which pairs of means from 4 different positions were significantly different for FA_{area} of *Q. alba.*

Position (A) Position (B)	Mean difference (A-B)	SE	*p* .
South outer vs. South inner	-0.047	0.017	0.041
South outer vs. North outer	0.001	0.016	1.000
South outer vs. North inner	-0.066	0.018	0.002
South inner vs. North outer	0.048	0.017	0.025
South inner vs. North inner	-0.019	0.016	1.000
North outer vs. North inner	-0.067	0.017	0.000

Table 12 Bonferroni pairwise comparisons test to determine which pairs of means from 4 different positions were significantly different for FA_{area} of *Q. velutina*.

Position (A) Position (B)	Mean difference (A-B)	SE	*p*-value
South outer vs. South inner	-0.022	0.019	1.000
South outer vs. North outer	-0.046	0.017	0.041
South outer vs. North inner	-0.041	0.021	0.299
South inner vs. North outer	-0.024	0.016	0.822
South inner vs. North inner	-0.019	0.016	1.000
North outer vs. North inner	-0.006	0.017	1.000

Table 13 Bonferroni pairwise comparisons test to determine which pairs of means from 4 different positions were significantly different for FA_{width} of *Q. alba*.

Position (A) Position (B)	Mean difference (A-B)	SE	*p*-value
South outer vs. South inner	-0.024	0.016	0.778
South outer vs. North outer	0.011	0.015	1.000
South outer vs. North inner	-0.035	0.016	0.179
South inner vs. North outer	0.035	0.015	0.143
South inner vs. North inner	-0.011	0.015	1.000
North outer vs. North inner	0.046	0.016	0.021

Table 14 Bonferroni pairwise comparisons test to determine which pairs of means from 4 different positions were significantly different for FA_{width} of *Q. velutina*.

Position (A) Position (B)	Mean difference (A-B)	SE	*p*-value
South outer vs. South inner	-0.041	0.016	0.066
South outer vs. North outer	-0.045	0.015	0.014
South outer vs. North inner	-0.058	0.017	0.004
South inner vs. North outer	-0.005	0.014	1.000
South inner vs. North inner	-0.018	0.014	1.000
North outer vs. North inner	-0.013	0.015	1.000

Table 15 Bonferroni pairwise comparisons test to determine which pairs of means from 4 different positions were significantly different for leaf area of *Q. alba.*

Position (A) Position (B)	Mean difference (A-B)	SE	*p*-value
South outer vs. South inner	-0.007	0.002	0.001
South outer vs. North outer	0.000	0.002	1.000
South outer vs. North inner	0.010	0.002	0.000
South inner vs. North outer	0.008	0.002	0.000
South inner vs. North inner	-0.002	0.002	0.698
North outer vs. North inner	0.001	0.002	0.000

Table 16 Bonferroni pairwise comparisons test to determine which pairs of means from 4 different positions were significantly different for leaf area of *Q. palustris.*

Position (A) Position (B)	Mean difference (A-B)	SE	*p*-value
South outer vs. South inner	-0.012	0.002	0.000
South outer vs. North outer	0.000	0.002	1.000
South outer vs. North inner	-0.013	0.002	0.000
South inner vs. North outer	0.011	0.002	0.000
South inner vs. North inner	-0.001	0.002	1.000
North outer vs. North inner	0.012	0.002	0.000

Table 17 Bonferroni pairwise comparisons test to determine which pairs of means from 4 different positions were significantly different for leaf area of *Q. velutina*.

Position (A)	Position (B)	Mean difference (A-B)	SE	*p*-value
South outer vs.	South inner	-0.034	0.004	0.000
South outer vs.	North outer	-0.019	0.003	0.000
South outer vs.	North inner	-0.040	0.004	0.000
South inner vs.	North outer	0.015	0.003	0.000
South inner vs.	North inner	-0.006	0.003	0.297
North outer vs.	North inner	0.021	0.003	0.000

Table 18 Bonferroni pairwise comparisons test to determine which pairs of means from 4 different positions were significantly different for leaf lobulation of *Q. alba*.

Position (A)	Position (B)	Mean difference (A-B)	SE	*p*-value
South outer vs.	South inner	0.323	0.421	1.000
South outer vs.	North outer	-0.541	0.350	0.740
South outer vs.	North inner	1.176	0.455	0.060
South inner vs.	North outer	-0.864	0.392	0.167
South inner vs.	North inner	0.853	0.348	0.087
North outer vs.	North inner	1.717	0.419	0.000

Table 19 Bonferroni pairwise comparisons test to determine which pairs of means from 4 different positions were significantly different for leaf lobing of *Q. palustris*.

Position (A)	Position (B)	Mean difference (A-B)	SE	*p*-value
South outer vs.	South inner	-0.006	0.001	0.000
South outer vs.	North outer	-0.004	0.001	0.007
South outer vs.	North inner	-0.004	0.001	0.027
South inner vs.	North outer	0.003	0.001	0.243
South inner vs.	North inner	0.002	0.001	0.284
North outer vs.	North inner	0.001	0.001	1.000

Table 20 Bonferroni pairwise comparisons test to determine which pairs of means from 4 different positions were significantly different for leaf shape of *Q. palustris*.

Position (A)	Position (B)	Mean difference (A-B)	SE	*p*-value
South outer vs.	South inner	0.010	0.005	0.318
South outer vs.	North outer	0.001	0.004	1.000
South outer vs.	North inner	0.016	0.005	0.013
South inner vs.	North outer	-0.009	0.005	0.371
South inner vs.	North inner	0.006	0.004	0.799
North outer vs.	North inner	-0.015	0.005	0.013

Table 21 Bonferroni pairwise comparisons test to determine which pairs of means from 4 different positions were significantly different for leaf shape of *Q. velutina*.

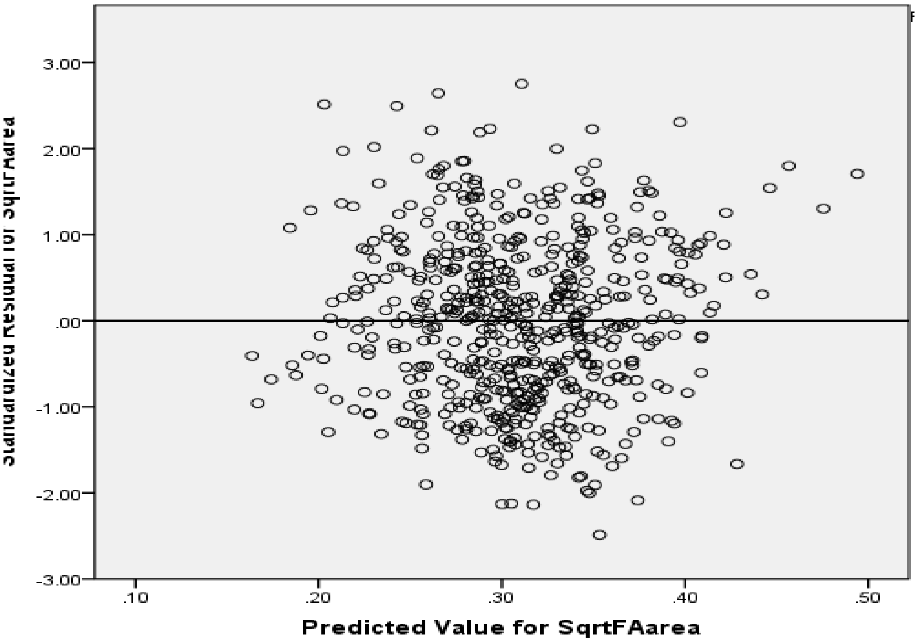
Position (A)	Position (B)	Mean difference (A-B)	SE	*p*-value
South outer vs.	South inner	0.008	0.002	0.001
South outer vs.	North outer	0.002	0.002	1.000
South outer vs.	North inner	0.009	0.002	0.001
South inner vs.	North outer	0.001	0.002	0.003
South inner vs.	North inner	0.003	0.002	1.000
North outer vs.	North inner	-0.008	0.002	0.001

APPENDIX F

Final Models for FA, Leaf Area, Lobulation and Shape for *Q. alba*

Table 22 Final general linear mixed model developed for *Q. alba* FA_{area} by square root transformation showing the sources of variation (Tree, N = 30; position, N = 4; leaf, N = 600; *p* = 0.05). Leaf was nested within position.

Source	Type III sum of square	df	Mean of Square	F	*p*
Intercept	0.609	1	0.609	32.986	0.000
Tree	0.802	29	0.028	1.499	0.047
Position	0.382	3	0.127	6.896	0.000
Leaf (Position)	0.117	16	0.007	0.396	0.983
M4	0.086	1	0.086	4.682	0.031
M5	0.214	1	0.214	11.589	0.001
M12	0.318	1	0.318	17.250	0.000
Error	10.113	548	0.018		.



. Figure 21 Residual plot of final model showing standardized residual for SqrtFAarea vs. predicted value for SqrtFAarea of *Q. alba*. The residual plot did not show any discernible pattern and most residuals were within 2 standard deviation from the mean, thus the model was accepted.

Table 23 Final nested ANOVA model developed for *Q. alba* FA_{width} by square root transformation showing the sources of variation (Tree, N = 30; position, N = 4; leaf, N = 600; *p* = 0.05).

Source	Type III sum of square	df	Mean of Square	F	*p*
Intercept	0.627	1	0.627	35.991	0.000
Tree	0.716	29	0.025	1.430	0.069
Position	0.182	3	0.061	3.514	0.015
Leaf (Position)	0.485	16	0.030	1.757	0.034
M5	0.414	1	0.414	23.967	0.000
M7	0.342	1	0.342	19.834	0.000
M11	0.168	1	0.168	9.728	0.002
Error	9.459	548	0.017		.

Table 24 Final nested ANOVA model developed for *Q. alba* leaf area by log transformation showing the sources of variation (Tree, N = 30; position, N = 4; leaf, N = 600; *p* = 0.05).

Source	Type III sum of square	df	Mean of Square	F	*p*
Intercept	0.005	1	0.005	26.621	0.000
Tree	0.023	29	0.001	4.570	0.000
Position	0.006	3	0.002	10.769	0.000
Leaf (Position)	0.004	16	0.000	1.313	0.183
M5	0.004	1	0.004	23.860	0.000
M7	0.003	1	0.003	14.434	0.000
M8	0.004	1	0.004	20.745	0.000
M9	0.002	1	0.002	10.064	0.002
M10	0.010	1	0.010	55.152	0.000
M11	0.006	1	0.006	34.423	0.000
M12	0.175	1	0.175	989.475	0.000
M14	0.002	1	0.002	9.070	0.003
M15	0.242	1	0.242	1371.650	0.000
Error	0.096	541	0.000		.

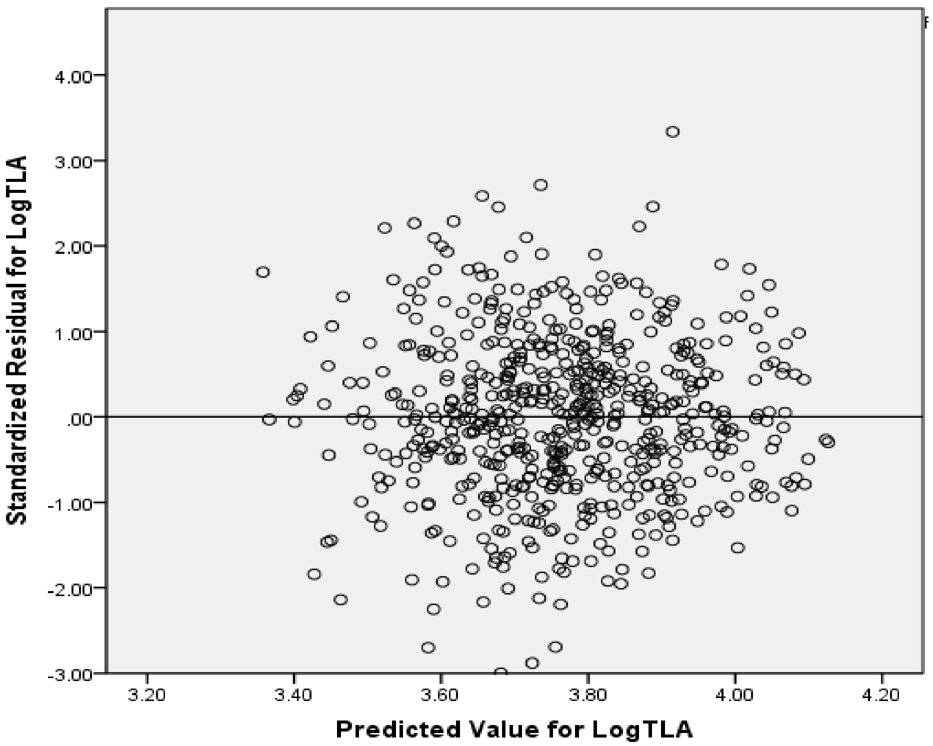


Figure 22 Residual plot of final model showing standardized residual for LogTLA vs. predicted value for LogTLA of *Q. alba*. The residual plot did not show any discernible pattern and most residuals were within 2 standard deviations from the mean, thus the model was accepted.

Table 25 Final nested ANOVA model developed for *Q. alba* leaf shape showing the sources of variation (Tree, N = 30; position, N = 4; leaf, N = 600; *p* = 0.05).

Source	Type III sum of square	df	Mean of Square	F	*p*
Intercept	3.698	1	3.698	2191.641	0.000
Tree	0.135	29	0.005	2.833	0.000
Position	0.001	3	0.000	0.203	0.894
Leaf (Position)	0.036	16	0.002	1.359	0.157
M2	0.642	1	0.642	390.253	0.000
M3	1.210	1	1.210	735.948	0.000
M5	0.246	1	0.246	149.523	0.000
M6	1.474	1	1.474	896.766	0.000
Error	0.889	547	0.002		.

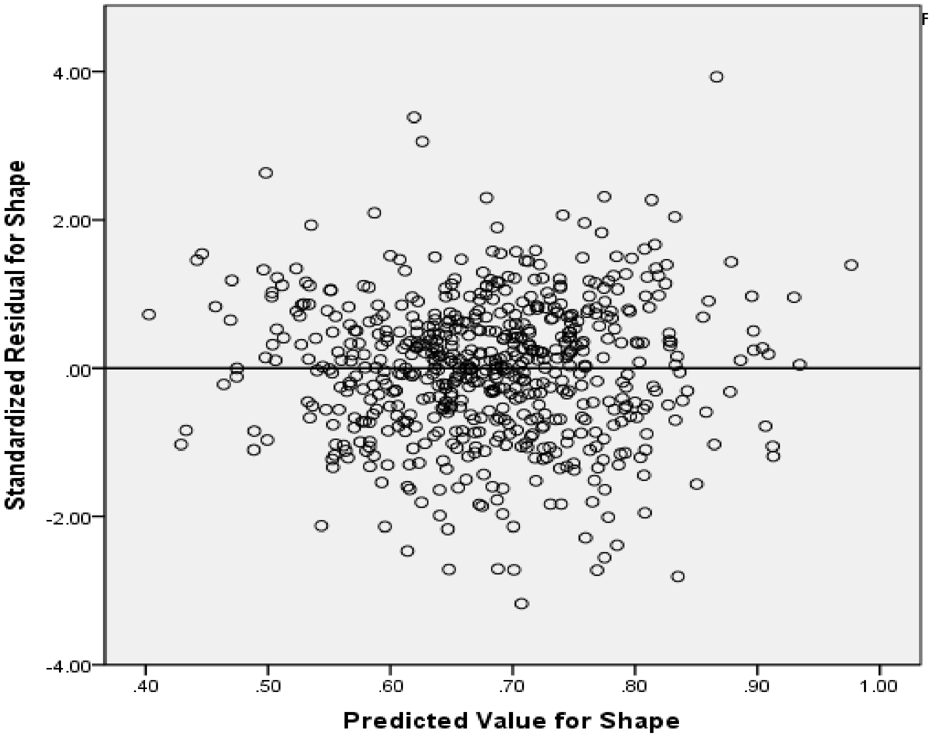


Figure 23 Residual plot of final model showing standardized residual for shape vs. predicted value for shape of *Q. alba*. The residual plot did not show any discernible pattern and most residuals were within 2 standard deviations from the mean, thus the model was accepted.

Table 26 Final nested ANOVA model developed for *Q. alba* leaf lobulation by square root transformation showing the sources of variation (Tree, N = 30; position, N = 4; leaf, N = 600; *p* = 0.05).

Source	Type III sum of square	df	Mean of Square	F	*p*
Intercept	1124.341	1	1124.314	127.623	0.000
Tree	4813.994	29	48.758	5.541	0.000
Position	151.441	3	50.480	5.736	0.001
Leaf (Position)	261.546	16	16.347	1.858	0.022
M3	269.036	1	269.036	30.572	0.000
M6	36.937	1	36.937	4.197	0.041
M10	1275.329	1	1275.329	144.922	0.000

Table 26 Continued

M12	3448.048	1	3448.048	391.820	0.000
M13	121.255	1	121.255	13.779	0.000
M14	4721.651	1	4721.651	536.546	0.000
Error	4798.045	545	8.800		.

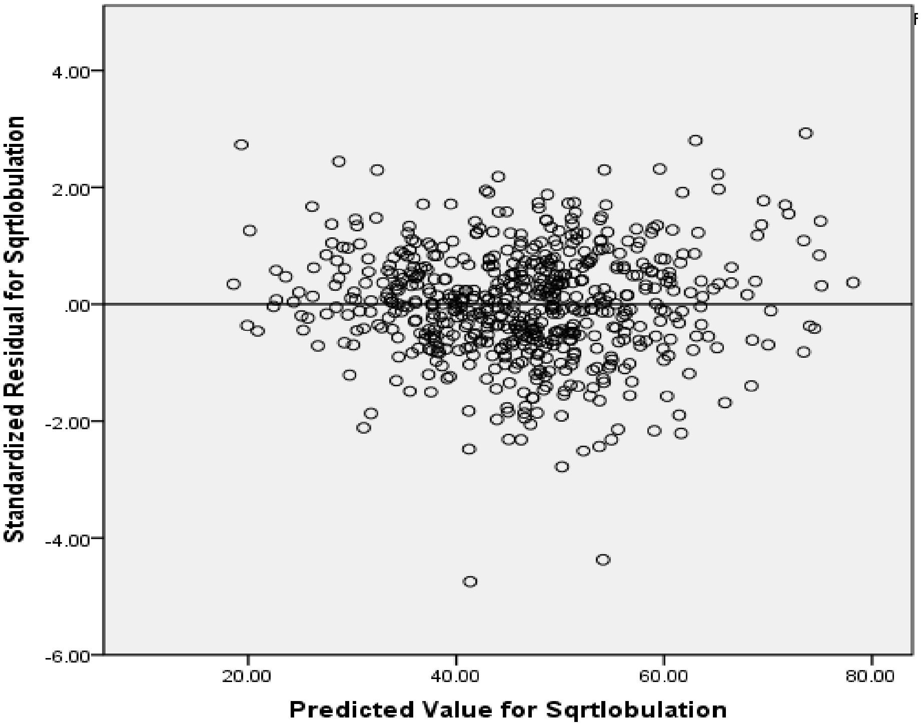


Figure 24 Residual plot of final model showing standardized residual for Sqrtlobulation vs. predicted value for Sqrtlobulation of *Q. alba*. The residual plot did not show any discernible pattern and most residuals were within 2 standard deviations from the mean, thus the model was accepted.

APPENDIX G

Final Models for FA, Leaf Area, Lobulation, and Shape for *Q. palustris*

Table 27 Final nested ANOVA model developed for *Q. palustris* FA_{area} by square root transformation showing the sources of variation (Tree, N = 30; position, N = 4; leaf, N = 600; p = 0.05).

Source	Type III sum of square	df	Mean of Square	F	*p*
Intercept	0.113	1	0.113	7.604	0.006
Tree	0.769	29	0.027	1.779	0.000
Position	0.028	3	0.009	0.619	0.603
Leaf (Position)	0.206	16	0.013	0.864	0.611
M5	0.085	1	0.085	5.678	0.018
M12	0.420	1	0.420	28.172	0.000
M13	0.177	1	0.177	11.866	0.001
Error	8.171	548	0.015		.

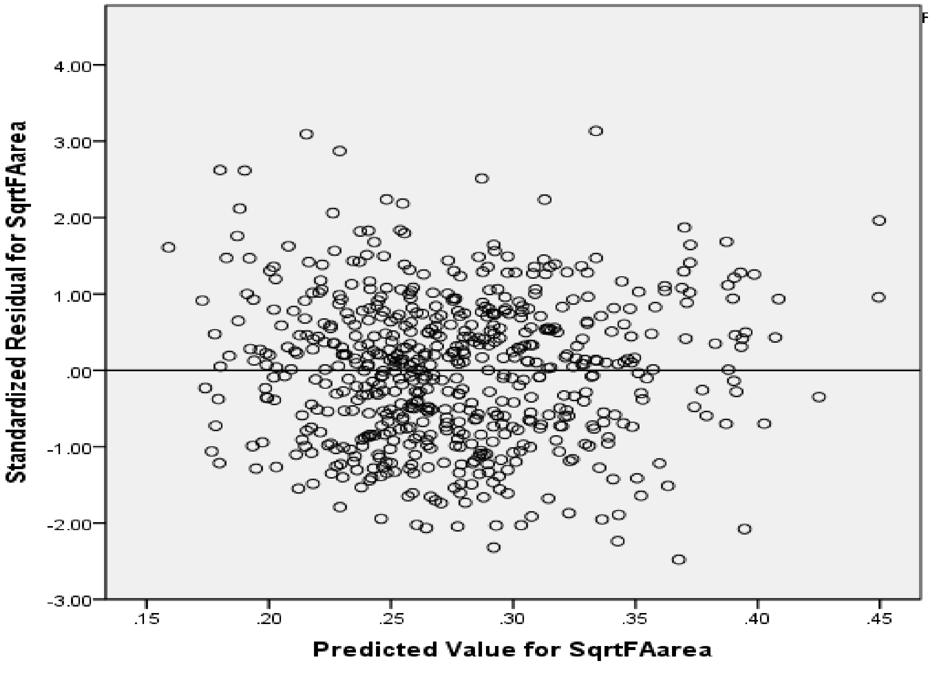


Figure 25 Residual plot of final model showing standardized residual for SqrtFAarea vs. predicted value for SqrtFAarea of *Q. palustris*. The residual plot did not show any discernible pattern and most residuals were within 2 standard deviations from the mean, thus the model was accepted.

Table 28 Final nested ANOVA model developed for *Q. palustris* FA_{width} by square root transformation showing the sources of variation (Tree, N = 30; position, N = 4; leaf, N = 600; *p* = 0.05).

Source	Type III sum of square	df	Mean of Square	F	*p*
Intercept	0.227	1	0.227	19.820	0.000
Tree	0.517	29	0.018	1.559	0.033
Position	0.022	3	0.007	0.633	0.594
Leaf (Position)	0.153	16	0.010	0.835	0.645
M2	0.087	1	0.087	7.619	0.006
M5	0.318	1	0.318	27.813	0.000
M6	0.089	1	0.089	7.780	0.005
M7	0.109	1	0.109	9.540	0.002
M10	0.045	1	0.045	3.951	0.047
Error	6.248	546	0.011		.

Table 29 Final nested ANOVA model developed for *Q. palustris* leaf area by log transformation showing the sources of variation (Tree, N = 30; position, N = 4; leaf, N = 600; *p* = 0.05).

Source	Type III sum of square	df	Mean of Square	F	*p*
Intercept	0.013	1	0.013	59.284	0.000
Tree	0.032	29	0.001	5.167	0.000
Position	0.008	3	0.003	13.025	0.000
Leaf (Position)	0.005	16	0.000	1.344	0.165
M4	0.003	1	0.003	12.201	0.001
M5	0.002	1	0.002	9.236	0.002
M6	0.005	1	0.005	21.755	0.000

Table 29 Continued

M7	0.004	1	0.004	19.217	0.000
M8	0.009	1	0.009	41.365	0.000
M11	0.003	1	0.003	15.257	0.000
M13	0.356	1	0.356	1686.017	0.000
M14	0.069	1	0.069	328.033	0.000
M15	0.008	1	0.008	36.618	0.000
Error	0.115	542	0.000		.

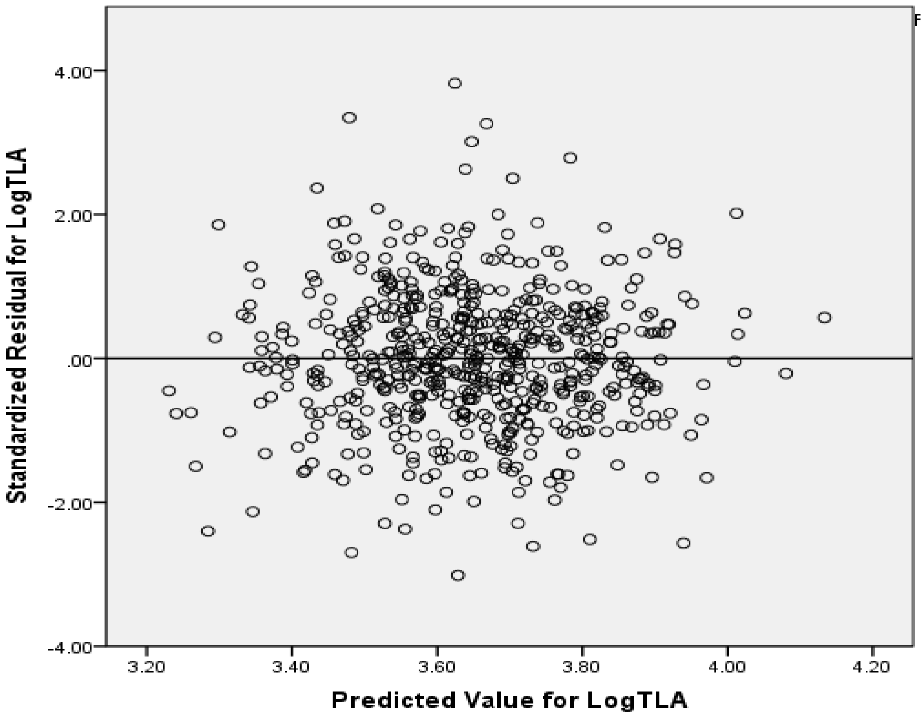


Figure 26 Residual plot of final model showing standardized residual for LogTLA vs. predicted value for LogTLA of *Q. palustris*. The residual plot did not show any discernible pattern and most residuals were within 2 standard deviations from the mean, thus the model was accepted.

Table 30 Final nested ANOVA model developed for *Q. palustris* leaf shape showing the sources of variation (Tree, N = 30; position, N = 4; leaf, N = 600; *p* = 0.05).

Source	Type III sum of square	df	Mean of Square	F	*p*
Intercept	1.492	1	1.492	1205.796	0.000
Tree	0.189	29	0.007	5.704	0.000
Position	0.013	3	0.004	3.789	0.010
Leaf (Position)	0.024	16	0.001	1.292	0.197
M1	0.006	1	0.006	5.207	0.023
M2	1.669	1	1.669	1462.706	0.000
M3	2.075	1	2.075	1817.929	0.000
M5	0.044	1	0.044	38.579	0.000
M6	1.021	1	1.021	894.344	0.000
M8	0.018	1	0.018	16.092	0.000
M15	0.281	1	0.281	246.596	0.000
Error	0.621	544	0.001		.

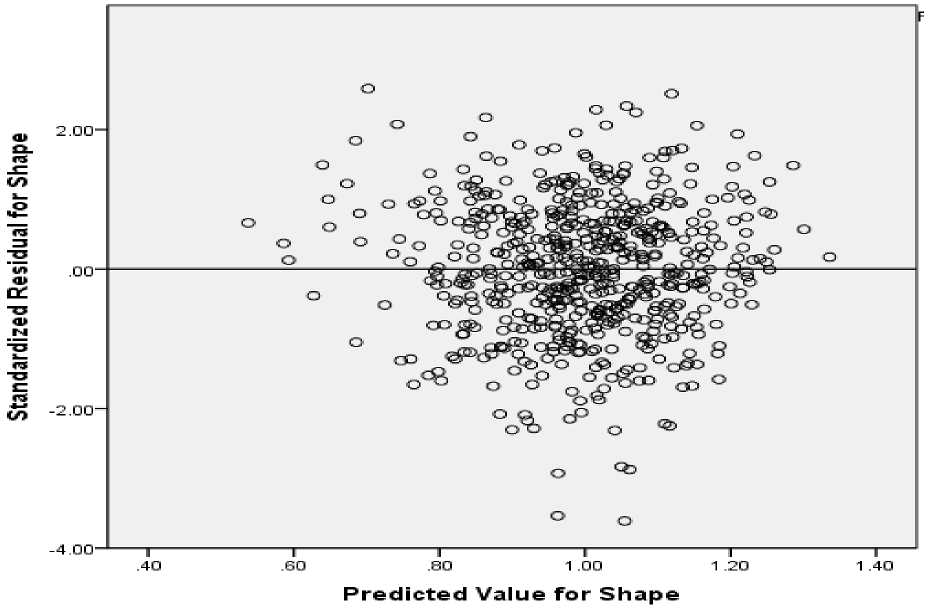


Figure 27 Residual plot of final model showing standardized residual for shape vs. predicted value for shape of *Q. palustris* The residual plot did not show any discernible pattern and most residuals were within 2 standard deviations from the mean, thus the model was accepted.

Table 31 Final nested ANOVA model developed for *Q. palustris* leaf lobulation by log transformation showing the sources of variation (Tree, N = 30; position, N = 4; leaf, N = 600; *p* = 0.05).

Source	Type III sum of square	df	Mean of Square	F	*p*
Intercept	0.001	1	0.001	8.213	0.004
Tree	0.010	29	0.000	4.477	0.000
Position	0.002	3	0.001	7.079	0.000
Leaf (Position)	0.001	16	0.000	0.792	0.695
M1	0.001	1	0.001	6.883	0.009
M5	0.000	1	0.000	3.850	0.050
M6	0.001	1	0.001	7.953	0.005
M10	0.001	1	0.001	17.455	0.000
M12	0.129	1	0.129	1633.591	0.000

Table 31 Continued

M13	0.092	1	0.092	1161.318	0.000
M14	0.460	1	0.460	5810.492	0.000
M15	0.409	1	0.409	5164.957	0.000
Error	0.043	541	7.924		.

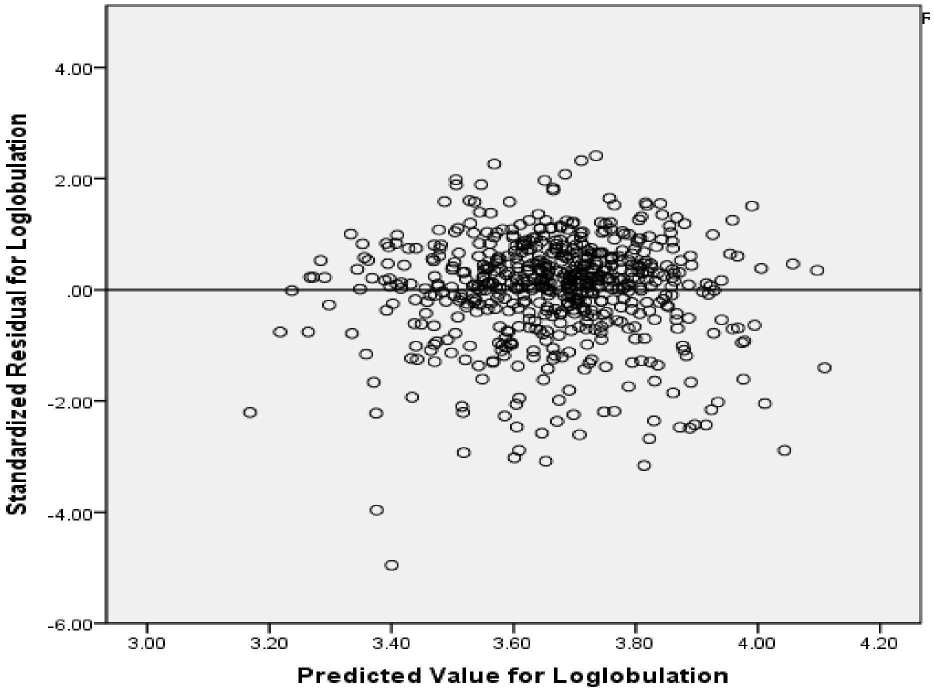


Figure 28 Residual plot of final model showing standardized residual for Loglobulation vs. predicted value for Loglobulation of *Q. palustris*. The residual plot did not show any discernible pattern and most residuals were within 2 standard deviations from the mean, thus the model was accepted.

APPENDIX H

Final Models for FA, Leaf Area, Lobulation, and Shape for *Q. velutina*

Table 32 Final nested ANOVA model developed for *Q. velutina* FA_{area} by square root transformation showing the sources of variation (Tree, N = 30; position, N = 4; leaf, N = 600; $p = 0.05$).

Source	Type III sum of square	df	Mean of Square	F	*p*
Intercept	0.298	1	0.298	16.141	0.000
Tree	1.189	29	0.041	2.224	0.000
Position	0.153	3	0.051	2.776	0.041
Leaf (Position)	0.371	16	0.023	1.259	0.219
M1	0.215	1	0.215	11.645	0.001
M3	0.130	1	0.130	7.049	0.008
M5	0.248	1	0.248	13.479	0.000
M9	0.120	1	0.120	6.499	0.011
M12	0.239	1	0.239	12.993	0.000
Error	10.063	546	0.018		.

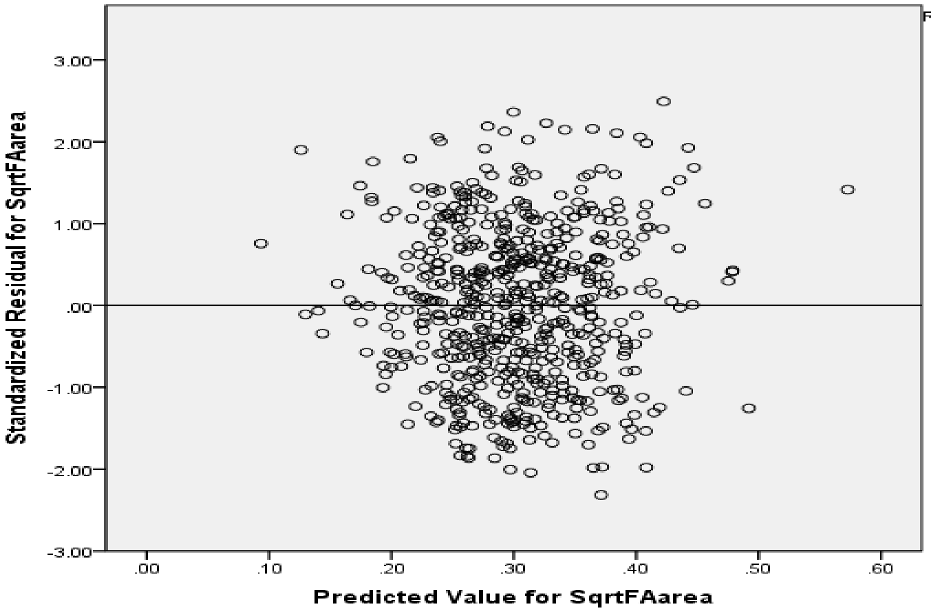


Figure 29 Residual plot of final model showing standardized residual for SqrtFAarea vs. predicted value for SqrtFAarea of *Q. velutina*. The residual plot did not show any discernible pattern and most residuals were within 2 standard deviations from the mean, thus the model was accepted.

Table 33 Final nested ANOVA model developed for *Q. velutina* FA_{width} by square root transformation showing the sources of variation (Tree, N = 30; position, N = 4; leaf, N = 600; *p* = 0.05).

Source	Type III sum of square	df	Mean of Square	F	*p*
Intercept	0.593	1	0.593	40.587	0.000
Tree	0.735	29	0.025	1.733	0.011
Position	0.199	3	0.066	4.532	0.004
Leaf (Position)	0.143	16	0.009	0.614	0.874
M3	0.066	1	0.066	4.483	0.035
M4	0.068	1	0.068	4.620	0.032
M5	0.347	1	0.347	23.707	0.000
M12	0.255	1	0.225	17.471	0.000

Table 33 Continued

M15	0.139	1	0.139	9.521	0.002
Error	7.981	546	0.015		.

Table 34 Final nested ANOVA model developed for *Q. velutina* leaf area by log transformation showing the sources of variation (Tree, N = 30; position, N = 4; leaf, N = 600; *p* = 0.05).

Source	Type III sum of square	df	Mean of Square	F	*p*
Intercept	0.007	1	0.007	10.196	0.001
Tree	0.311	29	0.011	16.199	0.000
Position	0.067	3	0.022	33.989	0.000
Leaf (Position)	0.012	16	0.001	1.123	0.330
M1	0.005	1	0.005	7.091	0.008
M2	0.034	1	0.034	51.488	0.000
M8	0.018	1	0.018	26.484	0.000
M9	0.009	1	0.009	14.155	0.000
M10	0.044	1	0.044	67.021	0.000
M11	0.024	1	0.024	36.771	0.000
M14	0.421	1	0.421	636.035	0.000
M15	0.553	1	0.553	836.027	0.000
Error	0.359	543	0.001		.

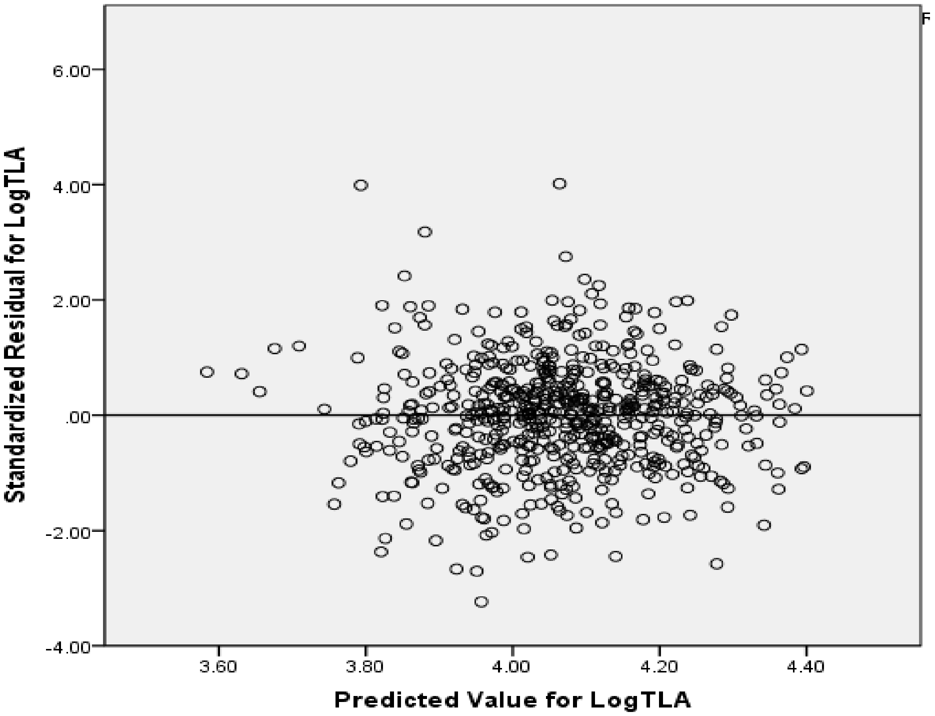


Figure 30 Residual plot of final model showing standardized residual for LogTLA vs. predicted value for LogTLA of *Q. velutina*. The residual plot did not show any discernible pattern and most residuals were within 2 standard deviations from the mean, thus the model was accepted.

Table 35 Final nested ANOVA model developed for *Q. velutina* leaf shape by log transformation showing the sources of variation (Tree, N = 30; position, N = 4; leaf, N = 600; *p* = 0.05).

Source	Type III sum of square	df	Mean of Square	F	*p*
Intercept	0.026	1	0.026	106.831	0.000
Tree	0.018	29	0.001	2.595	0.000
Position	0.006	3	0.002	7.920	0.000
Leaf (Position)	0.004	16	0.000	1.033	0.419
M2	0.229	1	0.229	950.131	0.000
M3	0.328	1	0.328	1365.190	0.000
M5	0.009	1	0.009	35.842	0.000
M6	0.206	1	0.206	857.612	0.000

Table 35 Continued

M12	0.006	1	0.006	24.535 0.000	0.000
M13	0.070	1	0.070	290.717	0.000
Error	0.131	544	0.000		.

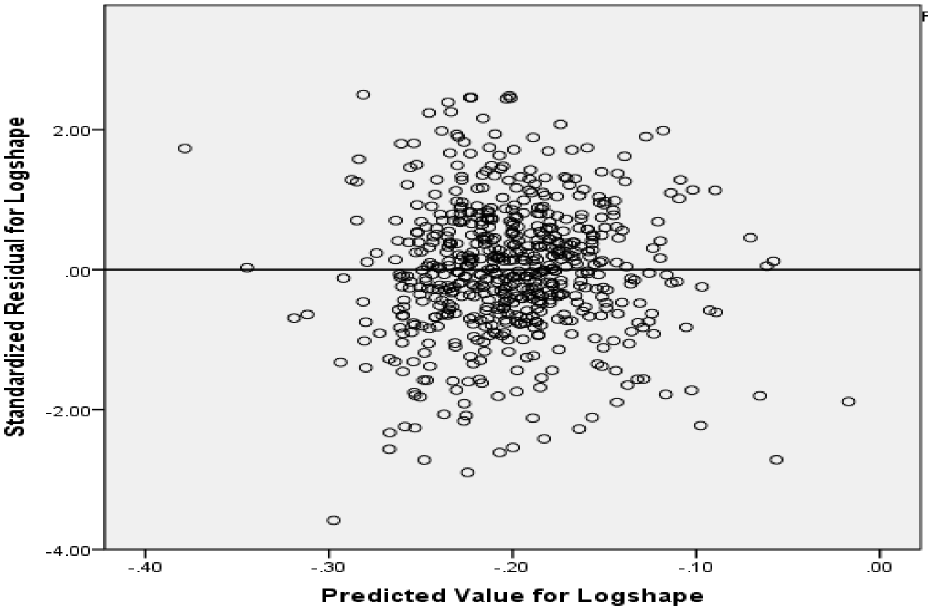


Figure 31 Residual plot of final model showing standardized residual for Logshape vs. predicted value for Logshape of *Q. velutina*. The residual plot did not show any discernible pattern and most residuals were within 2 standard deviations from the mean, thus the model was accepted.

Table 36 Final nested ANOVA model developed for *Q. velutina* leaf lobulation by square root transformation showing the sources of variation (Tree, N = 30; position, N = 4; leaf, N = 600; *p* = 0.05).

Source	Type III sum of square	df	Mean of Square	F	*p*
Intercept	5232.395	1	5232.395	949.027	0.000
Tree	761.829	29	26.270	4.768	0.000
Position	9.274	3	3.091	0.561	0.641
Leaf (Position)	60.173	16	3.761	0.683	0.812

Table 36 Continued

M1	89.587	1	89.587	16.260	0.000
M6	35.686	1	35.686	6.477	0.011
M8	46.117	1	46.117	8.370	0.004
M10	165.643	1	165.643	30.065	0.000
M12	3424.049	1	3424.049	621.478	0.000
M13	2098.761	1	2098.761	380.933	0.000
M14	6749.120	1	6749.120	1224.990	0.000
M15	4615.326	1	4615.326	837.699	0.000
Error	2991.675	543	5.5110		.

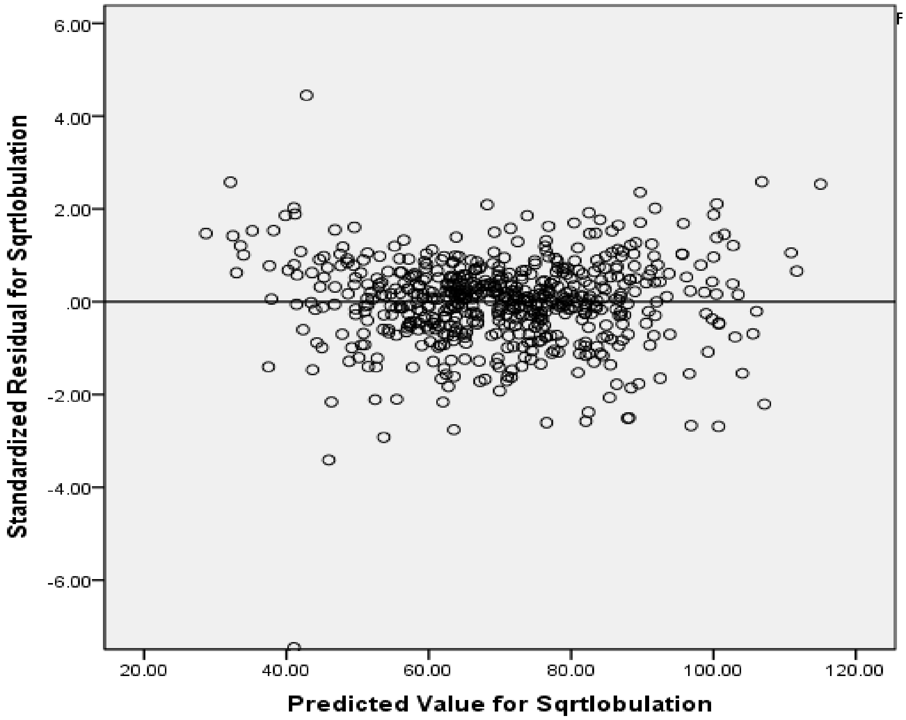


Figure 32 Residual plot of final model showing standardized residual for Sqrtlobulation vs. predicted value for Sqrtlobulation of *Q. velutina*. The residual plot did not show any discernible pattern and most residuals were within 2 standard deviations from the mean, thus the model was accepted.

APPENDIX I

ANOVA Designed by General Linear Model to Determine the Effects of Tree (N = 90) height and Diameter on FAarea of *Q. alba*, *Q. palustris*, and *Q. velutina*

Table 37 ANOVA designed by general linear model to determine the effects of tree (N =30) height and diameter on FA_{area} of *Q. alba*.

Source	Type III Sum of Squares	Mean Square	F	*p*
Corrected Model	0.005	0.003	2.823	0.077
Intercept	0.001	0.001	0.916	0.347
Logheight	0.000	0.000	0.024	0.879
Logdiameter	0.003	0.003	3.009	0.094
Error	0.026	0.001		

Table 38 ANOVA designed by general linear model to determine the effects of tree (N =30) height and diameter on FA_{area} of *Q. palustris*.

Source	Type III Sum of Squares	Mean Square	F	*p*
Corrected Model	0.003	0.002	1.232	0.308
Intercept	0.118	0.118	90.019	0.000
Height	0.000	0.000	0.005	0.945
Diameter	0.003	0.003	2.305	0.141
Error	0.035	0.001		

Table 39 ANOVA designed by general linear model to determine the effects of tree (N =30) height and diameter on FA_{width} of *Q. palustris*.

Source	Type III Sum of Squares	Mean Square	F	*p*
Corrected Model	0.001	0.000	0.601	0.555
Intercept	0.082	0.082	108.020	0.000
Height	0.000	0.000	0.410	0.527
Diameter	0.001	0.001	1.010	0.324
Error	0.020	0.001		

Table 40 ANOVA designed by general linear model to determine the effects of tree (N =30) height and diameter on FA_{area} of *Q. velutina*.

Source	Type III Sum of Squares	Mean Square	F	*p*
Corrected Model	0.000	0.000	0.128	0.880
Intercept	0.242	0.242	125.586	0.000
Height	0.000	0.000	0.233	0.633
Diameter	0.000	0.000	0.179	0.675
Error	0.052	0.002		

Table 41 ANOVA designed by general linear model to determine the effects of tree (N =30) height and diameter on FA_{width} of *Q. velutina*.

Source	Type III Sum of Squares	Mean Square	F	*p*
Corrected Model	0.000	0.000	0.131	0.878
Intercept	0.189	0.189	231.271	0.000
Height	0.000	0.000	0.224	0.640
Diameter	0.000	0.000	0.021	0.885
Error	0.022	0.001		

APPENDIX J

Minimum and Maximum Mean Value and Plasticity Indices of *Q. alba*, *Q. palustris*, and *Q. velutina*.

Table 42 Minimum and maximum mean values and plasticity index of *Q. alba*

	South outer	South inner	**Plasticity**	North outer	North inner	**Plasticity**
Traits	1	2		3	4	
M1	16.910000	14.42	**0.11**	17.35	14.53	**0.16**
M2	40.52	49.08	**0.17**	43.88	49.94	**0.12**
M3	88.74	96.23	**0.08**	94.73	98.53	**0.04**
M4	23.63	25.77	**0.08**	26.58	25.62	**0.04**
M5	58.74	64.56	**0.09**	62.47	66	**0.05**
M6	45.04	48.9	**0.08**	47.36	49.64	**0.05**
M7	44.14	48.55	**0.09**	46.93	49.68	**0.06**
M8	22.45	29.84	**0.25**	24.25	31.93	**0.24**
M9	13.99	17.64	**0.21**	14.85	18.97	**0.22**
M10	36.37	32.54	**0.11**	37.35	32	**0.14**
M11	13.34	12.7	**0.05**	14.08	12.19	**0.13**
M12	2492.32	3419.94	**0.27**	2809.69	3597.74	**0.22**
M13	2458.36	3411.66	**0.28**	2825.09	3700.44	**0.24**
M14	3627.86	4506.45	**0.19**	4086	4664.09	**0.12**
M15	3582.7	4522.33	**0.21**	4114.49	4747.94	**0.13**
TLA	4950.68	6831.6	**0.28**	5634.79	7298.18	**0.23**
LBL	129.26	145.31	**0.11**	138.61	148.47	**0.07**
TLL	146.17	159.73	**0.08**	155.96	163	**0.04**

Table 43 Minimum and maximum mean values and plasticity index of *Q. palustris*.

	South outer	South inner	**Plasticity**	North outer	North inner	**Plasticity**
Traits	1	2		3	4	
M1	40.89	47.99	**0.15**	45.38	47.54	**0.05**
M2	29.81	38.35	**0.22**	30.48	38.93	**0.22**
M3	78.83	87.94	**0.10**	83.69	88.57	**0.06**
M4	25.82	29.64	**0.13**	26.98	30.2	**0.11**
M5	62.47	71.7	**0.13**	67.77	71.91	**0.06**
M6	54.93	60.6	**0.09**	60.06	60.19	**0.00**

Table 43 Continued

M7	54.65	60.47	**0.10**	59.79	60.53	**0.01**
M8	13.3	18.88	**0.30**	13.73	18.99	**0.28**
M9	8.73	11.05	**0.21**	9.28	11.33	**0.18**
M10	36.71	41.31	**0.11**	40.49	41.35	**0.02**
M11	21.82	22.67	**0.04**	22.4	23.51	**0.05**
M12	1762.63	2798.83	**0.37**	2057.74	2796.13	**0.26**
M13	1767.11	2806.37	**0.37**	2087.75	2824.49	**0.26**
M14	3976.97	5364.47	**0.26**	4647.09	5373.98	**0.14**
M15	3892.56	5275.78	**0.26**	4616.95	5353.75	**0.14**
TLA	3529.74	5605.2	**0.37**	4145.5	5620.62	**0.26**
LBL	108.64	126.29	**0.14**	114.17	127.5	**0.10**
TLL	149.53	174.29	**0.14**	159.55	175.04	**0.08**

Table 44 Minimum and maximum mean values and plasticity index of *Q. velutina*.

	South outer	South inner	**Plasticity**	North outer	North inner	**Plasticity**
Trait	1	2		3	4	
M1	53.63	48.6	**0.09**	52.19	48.33	**0.07**
M2	44.82	55.42	**0.19**	52.21	59.01	**0.12**
M3	121.64	116.12	**0.05**	125.45	121.05	**0.04**
M4	37.27	37.72	**0.01**	38.3	40.11	**0.05**
M5	89.62	90.5	**0.01**	92.5	96.58	**0.04**
M6	69.41	68.84	**0.01**	71.08	73.21	**0.03**
M7	68.87	69.4	**0.01**	71.13	73.15	**0.03**
M8	32.16	40.16	**0.20**	37.16	41.93	**0.11**
M9	13.06	19.49	**0.33**	17.16	20.73	**0.17**
M10	45.82	35.84	**0.22**	40.93	35.33	**0.14**
M11	27.44	23.39	**0.15**	26.16	25.72	**0.02**
M12	5071.5	6110.27	**0.17**	6017.69	7133.5	**0.16**
M13	5100.64	6364.51	**0.20**	6026.43	7176.76	**0.16**
M14	8178.72	8352.15	**0.02**	8803.5	9495.89	**0.07**
M15	8158.27	8510.61	**0.04**	8707.93	9463.76	**0.08**
TLA	10172.14	12474.78	**0.18**	12044.12	14310.27	**0.16**
LBL	166.46	171.54	**0.03**	172.65	180.06	**0.04**
TLL	220.09	220.13	**0.00**	224.84	228.39	**0.02**

VITA

JOSEPH KUSI

Personal Data:	Place of Birth**:** Berekum, Ghana.
Education	MS Biology, East Tennessee State University, Johnson City, Tennessee, 2013 BSc. Environmental Sciences, University of Cape Coast, Ghana 2008 Teacher's Certificate 'A' St. Joseph's College of Education, Bechem, Ghana 2001
Experience	Teaching Associate, Department of Biological Sciences, East Tennessee State University, Johnson City, TN 2011-2013. Biology Tutor, Acherensua Senior High School, Acherensua, Ghana 2008-2010.
Awards and Grants	Graduate Research Council Award, The School of Graduate Studies, East Tennessee State University, Johnson City, Tennessee 2012 Marcia Davis Research Award for Graduate Research in Conservation of Natural Resources, Department of Biological Sciences, East Tennessee State University, Johnson City, Tennessee 2012

Conferences Attended	Appalachian Student Research Forum, East Tennessee State University, Johnson City, TN 2012 Appalachian Student Research Forum, East Tennessee State University, Johnson City, TN 2013
Technical Skills:	SPSS, ImageJ, Gel electrophoresis, dissection of mammals, microscopy, collection and pressing of leaves.