Biomechanical and morphological changes in leaf abscission zones during the ontogeny of Kalanchoe pinnatum

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and

The Honors Program

of

Butler University

Jillian Hodge

April 23, 2010
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Chapter 1: The properties and characteristics of leaf abscission

Leaf Function

Leaves are commonly recognized as the main photosynthetic organ of the plant. Chloroplasts within leaves capture the energy from sunlight and use it to transform this energy into food for the plant. Leaves also aid plants in gas exchange and transpiration which allows water to be drawn upwards through the plant as it evaporates from a leaf’s surface (Campbell and Reece, 2005). In many plants, however, leaves have also evolved other functions that allow plants to live in water, provide protection, or even allow them to asexually reproduce (Table 1).

Leaf Morphology

Just as leaves possess several different functions, leaves also exhibit different shapes or morphologies. Generally, plants display two main morphologies: simple or compound. In a simple leaf (Fig. 1A; Fig. 2A), the leaf is made of a single, complete blade. Though its margin (edges) might be deeply lobed, the body of the leaf remains undivided. A petiole then connects the leaf blade to a node on the plant’s stem (Campbell and Reece, 2005). In contrast, a compound leaf (Fig. 1b; Fig. 2b) contains a blade that is divided into multiple distinct leaflets. Each leaflet is connected to a common rachis by a small petiolule, and the entire rachis is then connected to the petiole. A leaflet is not considered a leaf as there is no axillary bud at its base, which allows for new leaf growth to occur (Campbell and Reece, 2005).

Heterophylly

Most plants exhibit a single leaf morphology with only simple leaves or only compound leaves. Other plant species, however, express the phenomenon of
heterophylly, meaning that different leaf morphologies are present on a single plant at different times or different positions (Fig. 3). In aquatic plants, for example, rounded, thick leaves covered in stomata are observed in dry conditions, whereas long, thin leaves with no stomata are formed when the plant is submerged (Cook, 1969). In English ivy (*Hedera helix*), leaves present on the climbing stems are palmately lobed, whereas leaves on the fertile flowering stems have unlobed cordate leaves (Metcalfe, 2005). Because leaf shape can influence leaf function, heterophylly may have a significant impact on plant fitness (Winn, 1999).

*Abscission*

Plant fitness is also affected by abscission, the process by which a plant loses organs such as fruit, flowers, or leaves (Gawadi and Avery, 1950). For example, unwanted plant organs such as old leaves or flower petals may be abscised when they no longer serve a function on the plant. Additionally, damaged or infected organs may be quickly abscised as a defense mechanism. Plant parts such as fruits or leaves may even be abscised to ensure future propagation success (Patterson, 2001).

Abscission occurs at a point of separation known as the abscission zone. Unlike adjacent tissue, the abscission zone is distinguished by its thin-walled, closely packed, and uniformly smaller cells (Fig. 4) (Gawadi and Avery, 1950). A leaf’s number and position of abscission zones differ among plant species, however, most simple leaves develop one abscission zone at the base of the petiole’s connection to the stem (Fig. 1A). Most compound leaves have an abscission zone at the base of each leaflet’s connection to the rachis, the petiolule, and an additional one at the base of the entire leaf’s petiole (Fig. 1B) (Gawadi and Avery, 1950).
When a mechanical stress like wind or rain causes leaf detachment, the actual break occurs in the separation layer, defined as one or more rows of cells in the abscission zone (Gawadi and Avery, 1950). Additionally, some plant species whose leaves abscise during harsh environmental conditions also develop an adjacent protective layer on the stem side (Fig. 5). This protective layer helps prevent bacteria or pathogens from entering the damaged area after leaf separation has occurred (Addicott, 1982).

Though the broad pattern by which abscission occurs is the same in all leaves, abscission characteristics vary greatly among different plant species. In deciduous plants, leaves are abscised before winter or a dry season, while evergreen plants continually shed their leaves throughout the year. In fruit-bearing plants, fruit is often abscised while still immature (Campbell and Reece, 2005). Abscission zones among plants can also be described as weak or strong. In species where leaf attachment is strong, abscission zones are said to be weak because the separation layer is poorly developed (Gawadi and Avery, 1950). Oak trees, for example, have weak abscission zones, resulting in tattered leaves that remain attached throughout the winter (Campbell and Reece, 2005). In species where leaves easily detach, however, the clearly defined abscission zone is said to be strong. Honey locusts, for example, have prominent abscission zones resulting in numerous leaves falling only moments after the lightest breeze (Campbell and Reece, 2005).

Specific Example: Kalanchoe pinnatum

Examining the properties and characteristics of leaves becomes especially interesting in the case of Kalanchoe pinnatum. K. pinnatum is a succulent plant native to Madagascar. Today, it is distributed in temperate regions of Australia, New Zealand, Macaronesia, Galapagos, and Hawaii (Beals, 1923). Commonly referred to as the “air
plant” or “miracle leaf,” *K. pinnatum* possesses a unique adaptation that allows the plant to produce tiny visible embryos embedded in the notches of a leaf’s margin (Kakesita, 1928). If the leaf falls due to an external disturbance such as wind or rain, the embryos begin to grow, (Fig. 6) eventually forming new individuals (Merlich, 1931). Thus, the leaves of *K. pinnatum* serve dual functions. In the absence of a disturbance, the leaves can stay on the plant and continue their role in photosynthesis. If an event removes a leaf from the plant, however, it will fall to the ground and begin reproducing asexually. This common occurrence allows *K. pinnatum* to create new individuals without the fertilization of gametes or structures such as flowers, fruits, or seeds. Though *K. pinnatum* can reproduce sexually in nature, it has not been observed in the plants grown in Butler University’s greenhouse (P.J. Villani, personal observation).

Whether a leaf stays on the plant or falls to the ground depends on how tightly attached the leaf is to the plant. Much more force will be required to remove a leaf that is firmly attached to a plant compared to a leaf that is loosely attached. Through personal observations, old leaves at the bottom of a *K. pinnatum* plant appear to require much more force to remove them than the young leaves at the top of the plant.

*K. pinnatum* leaf morphology and development

Examining the force required to remove a leaf from *K. pinnatum* requires consideration of both a leaf’s morphology and age. Like many other plants, *K. pinnatum* is heterophyllic expressing both simple and compound leaf morphologies simultaneously (Fig. 3). During initial growth of *K. pinnatum*, the apical meristem, or growing tip of undifferentiated tissue, produces juvenile leaves at the base of the plant. These leaves remain juvenile even as the stem continues to elongate and other leaves are produced.
Thus, leaves at the bottom of the plant are often old in age (meaning they were among the first on the plant) but still juvenile in terms of their phase development (Table 2). As *K. pinnatum* continues to grow into the adult phase, the apical meristem produces adult leaves. These leaves are young in age (meaning among the newest on the plant) but are adult with regard to their phase development (Flemming, 2005). In *K. pinnatum*, simple leaf morphology is present in juvenile phase leaves at the base of the plant and compound morphology is exhibited in adult phase leaves at the distal tip of the plant (Kakesita, 1928).

**Abscission in *K. pinnatum***

During the life of *K. pinnatum*, the likelihood of a leaf staying attached to the plant may not remain constant. Differences in leaf age and leaf morphology might mean there is a point in the leaf's development where abscission occurs more readily. When a leaf in *K. pinnatum* is abscised, the point of detachment is a separation layer in an abscission zone that forms near the base of the petiole (Taylor and Whitelaw, 2001). As the abscission layer in leaves matures, the contrast between these abscission layer cells and normal petiole cells appear much more prominent (Gawadi and Avery, 1950). As discussed earlier with leaves in general, the position and number of abscission zones also varies with respect to leaf morphology. Though these abscission properties have been described in multiple species, little is known about the abscission properties of *K. pinnatum* and how they vary among leaves of different age and morphology.

I suggest that properties of the abscission layer may vary both macroscopically and microscopically among leaves of different age and different morphology. In this study, I examine if the mechanical strength of petiole attachment and the microscopic
anatomy of the abscission zone vary during the life history of *K. pinnatum*. Investigating this difference might help determine if there is a point in the leaf’s development where functional demands change from photosynthesis to asexual reproduction.
Literature Cited


Figure 1: Schematic drawing of A) simple leaf and B) compound leaf depicting structures. Source: www-saps.plantsci.cam.ac.uk.
Figure 2. Photograph of A) simple leaf and B) compound leaf of *Kalanchoe pinnatum* depicting basic structures.
Figure 3. Heterophyllic leaf expression in *Kalanchoe pinnatum*. A) shows the simple leaves among the first to arrive on the plant and B) depicts the compound leaves that arrive later in the plant's life. The white marks on the stem between A) and B) represent the 9 leaf nodes whose leaves have been removed for better picture quality.
Figure 4. Diagram of abscission zone. A) defines the parts of a plant showing an abscission zone. The abscission zone is characterized by several layers of uniformly small cells compared to the petiole. B) depicts an abscission zone located between the two white lines in *Kalanchoe pinnatum*. 
Figure 5: Protective layer in base of the petiole of a maple. Some plant species develop a protective layer adjacent to the abscission layer on the stem side. This protective layer helps keep bacteria and pathogens from entering the plant after abscission has occurred. The abscission layer in the figure is equivalent to the separation layer. Source: http://openlearn.open.ac.uk.
Figure 6: Growing embryos of *Kalanchoe pinnatum* on a leaf that has abscised from the plant. The leaves of *K. pinnatum* have tiny embryos embedded in the notches of a leaf. These embryos then grow into new plants when the leaf falls to the ground.
Table 1. Examples of different adaptations leaves have evolved that allow plants to live in different environments (Campbell and Reece, 2005).

<table>
<thead>
<tr>
<th>Species Name</th>
<th>Common Name</th>
<th>Leaf Modification Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Carnegiea gigantean</em></td>
<td>Saguaro cactus</td>
<td>Spines offer protection</td>
</tr>
<tr>
<td><em>Dionaea muscipula</em></td>
<td>Venus Fly Trap</td>
<td>Leaves trap insects to help plant absorb nitrogen</td>
</tr>
<tr>
<td><em>Nymphaea leibergii</em></td>
<td>Dwarf Water-lilly</td>
<td>Waxy cuticle helps plant avoid water excess</td>
</tr>
<tr>
<td><em>Eucalyptus angustissima</em></td>
<td>Eucalyptus</td>
<td>Leaves emit poisons to deter herbivores</td>
</tr>
<tr>
<td><em>Kalanchoe pinnatum</em></td>
<td>Air – plant</td>
<td>Leaves fall to ground and allow plant to asexually</td>
</tr>
</tbody>
</table>
Table 2. Comparison of the terms associated with leaf age and phase development (Campbell and Reece, 2005).

<table>
<thead>
<tr>
<th>Picture</th>
<th>Leaf Phase (morphology)</th>
<th>Leaf Maturity</th>
<th>Temporal Age (time spent on plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Simple leaf" /></td>
<td>Simple</td>
<td>Juvenile</td>
<td>Oldest on Plant</td>
</tr>
<tr>
<td><img src="image" alt="Compound leaf" /></td>
<td>Compound</td>
<td>Adult</td>
<td>Youngest on Plant</td>
</tr>
</tbody>
</table>
Chapter 2: The properties of leaf abscission in *Kalanchoe pinnatum*

Abstract

*Kalanchoe pinnatum* is able to asexually reproduce with the help of its leaves. In *K. pinnatum*, embryos are embedded in the notches of a leaf’s margin. Through the process of abscission, when the plant is disturbed a leaf with embryos falls to the ground and the embryos grow into new plants. Thus, as leaves mature, they face conflicting functional demands to stay on the plant and continue their role in photosynthesis or fall off the plant and asexually reproduce. To examine if there is a point in the leaf’s development where abscission occurs more readily, I examined breaking strength in leaves that varied in age and morphology. I hypothesized as leaves mature in both developmental phase and temporal age, it would take less breaking strength to remove them from the plant and that the leaves’ tissues would reflect these mechanical changes. Results showed that temporal age and leaf morphology influenced breakage as well as the direction of force application. Though temporal age did not significantly affect mechanical properties beyond the first week, breaking strength varied most noticeably with phase development as compound leaves detached more easily than simple leaves. Additionally, leaf abscission in *K. pinnatum* will likely only easily occur when shear forces are exerted on the leaf compared to tensile forces.

Introduction

Different plant growth habits can be characterized by variations of mechanical properties during ontogeny (Gallenmuller et al., 2001). In lianas, for example, young branches are very stiff and rigid, allowing for them to be self-supportive. As these branches mature, however, they become more flexible and benefit from attaching themselves to new hosts (Rowe and Speck, 1996). Thus, as the mechanical properties of the lianas change, so do the branches’ function. Young branches change from being self-supportive to climbing branches (Rowe and Speck, 1996).

This same phenomenon occurs in *Kalanchoe pinnatum*. The leaves of *K. pinnatum* possess a special adaptation where tiny embryos are embedded in the notches of leaf margins (Kakesita, 1928). Unlike plants that utilize sexual reproduction, where the fertilization of gametes produces new individuals, *K. pinnatum* uses its specialized leaf adaptation to clone itself through asexual reproduction. In *K. pinnatum*, through the
process of abscission, a leaf with embryos falls to the ground and the embryos grow into new plants (Kakesita, 1928). Thus, as leaves mature, they face conflicting functional demands to remain on the plant and continue their role in photosynthesis, or, in the case of a disturbance, fall off the plant and asexually reproduce. In this study, I investigate if there is a point in the development of a leaf where abscission occurs more readily. This question can be examined on both macroscopic and microscopic levels to see if both the mechanical force needed to remove a leaf of *K. pinnatum* varies among leaves of different age and morphology and if the properties of the abscission zone correspond with the mechanical change.

The amount of mechanical force needed to remove a leaf from a plant can be measured by a biomechanical test called breaking strength. In this study, breaking strength (N/m²) is defined as the force needed to break a petiole from the main body of the plant divided by the cross-sectional area of the petiole (Vogel, 1980). While the breaking strength of leaves of *K. pinnatum* has not been measured, similar biomechanical tests using strain-guage force plates have been used to successfully measure the force exerted on limpets by individual waves (Denny, 1983). Although not directly comparable, the study shows that biomechanical tests are valid ways of examining the force of attachment.

While breaking strength is a direct measure of the force of attachment, microscopic techniques can also be used to examine how well attached a leaf is to the stem. Examination of the abscission zone can provide qualitative evidence of attachment strength. In particular, the diameters of cells in both the abscission zone and the petiole can be examined to see if differences occur in leaves varying in age and morphology.
Leaves with a well-defined abscission zone will exhibit cell sizes much smaller than adjacent cells in the petiole, whereas leaves with a weak abscission zone will not exhibit these same size differences.

The objective of this research is to examine whether the breaking strength of the abscission layer varies during the life history of *K. pinnatum*. I will examine this question on a macroscopic level to see if the mechanical force needed to remove a leaf of *K. pinnatum* varies among leaves of different age and morphology, and I will use microscopic techniques to see if changes in the abscission zone correspond to mechanical changes. When a leaf first appears on a plant, its primary function is photosynthesis suggesting that it would be advantageous to remain tightly attached to the plant. As a leaf ages over time, however, additional leaves appear on the plant reducing the importance of any single leaf as a photosynthetic organ. *K. pinnatum* is heterophyllic expressing both simple leaves at the bottom of the plant and compound leaves at the distal end. I suggest that it may no longer be as important to keep any single leaf as tightly attached. Thus, I hypothesize that the breaking strength of a simple leaf will decrease over time.

Additionally, as leaf morphology changes from simple to compound, it may be advantageous for the leaflets of the compound leaf or the compound leaf itself, to be easily detached from the plant as a mechanism of asexual reproduction.

**Research Methodology**

*Objective 1: Development of a single node over time*

My first objective was to determine if abscission properties (both mechanical and anatomical) varied at a single leaf node of *K. pinnatum* over a 5 week time period.
K. pinnatum specimens

Plants were asexually propagated in a growth chamber at room temperature under a 24 hour light cycle at Butler University beginning in May, 2009. Once the embryos had developed one complete set of leaves, they were transplanted to individual growing pots and placed in the Butler University greenhouse where they remained throughout the study. Because plants were asexually propagated, all of the plants were genetically identical. The plants were grown until the 5th leaf from the plant's base (5th node) was fully mature (Fig. 1). Leaves were defined as mature when no further increases were observed in leaf length over a 24 hour period (P.J. Villani, personal observation). I chose to study the 5th node because of its simple leaf morphology (compared to a compound morphology) and because it was neither the oldest nor youngest leaf on the plant. Because all plants were initially propagated on the same day, I was able to observe differences in leaf development by examining leaves from the 5th node on consecutive weeks. In this study, I compared a leaf on the 5th node once it had fully developed (referred to as Week 1) to that same node on a different plant at Week 2, 3, 4, and 5.

Mechanical Analysis

Tensile breaking strength is the maximum tensile load a leaf can withstand before breaking away from the plant (Vogel, 1988). I measured tensile breaking strength with a 25 Newton Pesola spring scale (Pesola AG, Baar, Switzerland) that was attached to an alligator clamp which was clamped onto the middle of the leaf's blade (Fig. 2). To ensure that the clamp would not slip from the leaf's waxy surface, a small piece of paper towel was inserted between the leaf and the clamp. Once the spring scale was in position, it was pulled perpendicularly to the stem until breakage occurred. After breakage, a razor
blade was used to cut a cross section of the petiole. The cross-sectional area of the petiole was measured through computer analysis of a micrograph of the removed petiole using SPOT imaging system (Daignostic Instruments, Sterling Heights, Michigan, USA). Tensile breaking strength (N/m²) was calculated by dividing the attachment force by the cross-sectional area of the petiole (Vogel, 1988).

**Microscopy**

In *K. pinnatum*, two leaves are situated on either side of the stem at each node (known as opposite phyllotaxy). The mechanical measurements were taken on one leaf and the petiole of the opposite leaf was collected for microscopy. For the microscopy samples, I used a razor blade to cut a 3mm long section where the petiole attached to the stem. This sample was prepared using standard histological techniques. It was preserved overnight in 2% gluteraldehyde, dehydrated with a graded ethanol series, infiltrated with a plastic embedding medium, sectioned on a microtome, stained with 0.05% toluidine blue, and observed under a light microscope. I then examined the cells between the petiole and the stem to note the presence or absence of an abscission zone. The abscission zone was characterized by a few rows of closely packed cells that appeared much smaller than adjacent cells. At 100x magnification, the abscission zone gave the general appearance of a faint line crossing the entire base of the petiole (Fig. 3). The abscission zone was described qualitatively by examining and comparing micrographs of different leaves. The abscission zone was also described quantitatively by averaging the measured diameter of ten cells in the abscission zone and ten cells in the petiole to determine if differences in cell sizes were significant. These microscopy techniques were learned during the Butler Summer Institute in Summer 2009.
Objective 2: Simple leaf vs. compound leaf

My second objective was to see if abscission properties (both mechanical and anatomical) varied between simple leaves (data collected from BSI Summer 2009) and compound leaves (data collected from Fall 2009) of *K. pinnatum*.

*K. pinnatum specimens*

The same plants grown during BSI Summer 2009 were maintained through the Fall of 2009. Plants were allowed to grow until they had both mature simple and compound leaves.

*Mechanical analysis*

Both tensile breaking strength (force applied perpendicularly to the stem) and shear breaking strength (force applied parallel to the stem) were measured on compound leaves to determine the amount of force required to remove both the entire compound leaf as well as an individual leaflet from the plant. These tests were only conducted on fully mature leaves. Maturity of compound leaves was determined when no further increases were observed in leaflet length over a 24 hour period (P.J. Villani, personal observation).

Tensile force was applied to both a side leaflet and an entire compound leaf to resemble the force that would be applied by an herbivore pulling perpendicularly on a leaf blade to detach the petiole from the stem (Vogel, 1988). A side leaflet was first removed by attaching the alligator clamp of a smaller 600g Pesola spring scale to the middle of a side leaflet (Fig. 2). The use of a paper towel for holding purposes was not needed for this test as the alligator clamp’s grip did not slip on the leaflet as it did on the simple leaf blade. Once the spring scale was placed, it was pulled perpendicularly to the rachis, and the force of detachment was normalized by dividing the cross-sectional area
of the petiolule, the connection between the base of the leaflet and the rachis of the compound leaf. Digital calipers were used to measure the major and minor axes of the petiolule and the cross sectional area was derived using the standard formula for the area of an ellipse. The same procedure was then used for the tensile breaking strength test of an entire compound leaf. The alligator clamp of the 1000g Pesola spring scale was attached to the middle of the front leaflet, and the leaflet was pulled perpendicularly to the stem. The force of detachment of the compound leaf petiole was again normalized by dividing by the cross-sectional area of the petiole as derived from the ellipse formula. Data from the compound leaves was compared to the simple leaves from objective 1.

A shear force was also applied to both an individual leaflet and the entire compound leaf to resemble the force of a brushing motion caused by an outside stimulus such as a gust of wind or a moving herbivore. Applying the force to the side leaflet was done by attaching a 600g Pesola spring scale (Pesola AG, Baar, Switzerland) with an S-hook onto the petiolule (Fig. 4). When applying the force to the entire compound leaf, the same S-hook scale was hung at the rachis. The rachis was supported by two fingers to ensure that proper breakage would occur at the rachis. The shear breaking strength was then measured by applying a downward force parallel to the stem. The force of detachment was again normalized by dividing by the cross-sectional area of the petiole derived from the ellipse formula (Vogel, 1988).

**Microscopy**

The same microscopic techniques used in the histological analysis of the simple leaf morphology (Summer 2009) were employed to examine abscission layers in both compound leaves and compound side leaflets.
Data analysis

All statistical analyses were calculated using Microsoft Excel Software (Version 2007). Direct comparisons of differences in tensile and shear breaking strength in compound leaves and tensile and shear breaking strength in compound side leaflets were conducted with paired t-tests. Paired t-tests were also used to compare cell sizes in the abscission zone and the petiole. Comparisons between tensile breaking strength in simple leaves over a five week period and comparisons in the tensile breaking strength of simple leaves, compound leaves, and compound side leaflets were conducted using a one-way analysis of variance (ANOVA) and Tukey's test for post-hoc comparisons.

Results

Results showed that developmental age, leaf morphology, and direction of force application influenced breakage.

Temporal Age

Examining the 5th node over a five week period showed that tensile breaking strength differed among weeks (Fig. 5). Between Week 1 and Week 2, there was a significant decrease in mean tensile breaking strength ($t=3.11, p=0.0055$). However, this trend was not carried on in subsequent weeks, as mean tensile breaking strength measured in Weeks 3-5 did not change significantly from the values measured during Week 2 ($df=4, F=3.86, p=0.0176$).

This result indicates that leaves at the fifth node were attached most tightly to the plant when they had just matured (Week 1 leaves), but the only significant decrease in attachment occurred between Week 1 and Week 2 as attachment values at
compared to leaves at Week 2, but that the attachment at Weeks 3-5 did not significantly differ from the attachment at Week 2.

Leaf morphology

Figure 6 shows that average tensile breaking strength varied significantly among simple leaves, compound leaves, and compound side leaflets (df=23, F=53.67, p<0.001). Post hoc comparisons using the Tukey test showed that the tensile breaking strength in simple leaves was significantly greater than either compound leaves (t=8.76, p<0.001) or compound side leaflets (t=10.13, p<.001), indicating that simple leaves are attached more tightly than either compound leaves or leaflets. The average tensile breaking strength of compound leaves was also greater than side leaflets, however, at the 0.05 level this difference is only marginally significant (df=9, t=1.80, p=0.0860). As with the tensile breaking strength, both compound leaves and side leaflets had shear breaking strength differences (Fig. 7) that were somewhat significant at the 0.05 level (df=9, t=2.09, p=0.0667). Together, these results indicate that compound leaves are possibly attached more tightly than individual leaflets.

Direction of force

In compound leaves, tensile breaking strength was significantly higher than shear breaking strength (Fig. 7) (df=9, t=4.47, p=0.0016). Tensile breaking strength was also significantly higher than shear breaking strength in the compound side leaflets (Fig. 7) (df=9, t=8.19, p<0.00001). This result indicates that in both compound leaves and side leaflets, petioles detached with tensile force were much harder to remove than petioles detached with shear force. Shear breaking strength was not measured among simple leaves, so I did not compare tensile to shear breaking strength.
Histology

Changes in the anatomy of *K. pinnatum* appear to be associated with the observed differences in mechanical tests. Figure 3 shows samples of abscission zones from simple leaves, compound leaves, and compound leaflets. Qualitatively, noticeable abscission zones were recognized in both the compound leaf (Fig. 3B) and the compound leaflet (Fig. 3C) by the presence of several tightly packed layers of uniformly small cells located in the area where the petiole attaches to the stem or the petiolule attaches to the rachis. Simple leaves (Fig. 3A), however, did not present a noticeable abscission zone as no visual distinction could be made between cells in the petiole and cells in the stem.

The abscission zones were also examined quantitatively among samples of simple leaves, compound leaves, and compound leaflets to see if cell sizes in the described abscission zone were significantly smaller than cell sizes in the petiole or petiolule (Table 1). In compound side leaflets, the difference in cell sizes was highly significant (df=29, t=11.07, p<0.001), as was the difference in cell sizes found in compound leaves (df=29, t=17.15, p<0.001). In simple leaves, the difference size between cells in the abscission zone and cells in the petiole was significant at a 0.05 level (df=29, t=2.6, p=0.02).

Discussion

Temporal age of leaves

As hypothesized, the old, simple leaves had much higher breaking strengths than young, compound leaves. Early in the plant’s life, it may be more critical for the leaf to be used in photosynthesis, making abscission from the plant highly unfavorable. However, later in life, when the plant has matured, and many leaves are present, leaf abscission may be highly advantageous as a mechanism of asexual propagation. The
plant may benefit from having the first leaves to appear on the plant remain firmly attached for photosynthetic purposes, while other mature leaves can be more easily removed from the plant to aid in asexual reproduction.

*Differences in simple vs. compound leaves*

As the leaves of *K. pinnatum* mature, they face conflicting functional demands to stay on the plant and continue their role in photosynthesis or fall off the plant and asexually reproduce (Mehrlich, 1931). In both compound leaves and compound side leaflets, breaking strength was much lower than simple leaves. This change in leaf morphology might mean that not only is there is a distinct change in the plant’s development (juvenile to mature), but that the function of a leaf changes as well. With simple leaf morphology, an entire leaf must stay attached for photosynthesis, or be dropped if used in asexual reproduction. However, with the compound leaf morphology, an individual leaflet can be easily detached and aid in reproduction while the main portion of the leaf remains attached and continues to photosynthesize. So, individual leaves may have dual purposes, or as my results suggest, subunits, such as a leaflet, may be specialized for individual functions.

*Differences between tensile vs. shear strength*

The significantly lower shear breaking strength compared to tensile breaking strength showed that the force needed to cause abscission was also dependant on the direction of force application. Tensile breaking strength examined the force required to remove the petiole perpendicularly from the stem as the applied force that pulled the petiole away from the plant directly opposed the resistant force of the stem (Vogel,
1988). While this is an interesting test to examine the force of attachment, it likely does not accurately depict natural petiole breaking tendencies, even in the case of herbivory.

Unlike tensile breaking strength, shear breaking strength tests examined the force required to remove the petiole in a different manner. Instead of applying force perpendicularly to the stem, shear forces were applied parallel to the stem (Vogel, 1988). This force did not pull the petiole directly away from the plant, but rather slid the petiole tissue away from the stem. Shear strength is a more biological relevant measure of attachment forces in *K. pinnatum* as it resembles breakage that would likely occur from stimuli in the environment, such as a moving herbivore brushing against a plant’s leaves as it passes.

*Anatomical/mechanical correlation*

Small changes in the anatomy of *K. pinnatum* may greatly influence the mechanical properties of the petiole. The appearance of a well-developed abscission layer, as shown by much smaller cells where the petiole attaches to the stem (Fig. 3b; Fig. 3c), may be associated with the decreased force needed to remove a leaf from the plant. While a well-developed abscission zone was found in mature compound leaves and compound side leaflets, the lack of a protective layer suggests that breakage does not occur at a set time, as seen in deciduous trees whose leaves fall off during a particular season. Rather, the likelihood of a leaf breaking off of the stem appears to increase over time, but may or may not occur depending on the external circumstances present in the environment. This is especially important as *K. pinnatum* is indigenous to a relatively stable, tropical environment where environmental changes are not likely to occur (Campbell and Reece, 2005). Abundant leaf abscission is not necessarily required for this
species to survive, but it is a phenomenon that simply seems to occur more easily at certain times during the plant's life.

Despite these interesting conclusions, several unanswered questions need to be addressed. This study investigated how abscission characteristics varied among leaves of different ages and different morphologies. When examining leaf age, a single node was examined over a 5 week period in which breaking strength varied significantly only between the first and second week, and the anatomy of the leaf did not appear to change qualitatively and only somewhat quantitatively. Examining these aspects on a weekly basis, however, might be somewhat arbitrary with regard to the plant's entire development. Assessment of maturity may also not match the reality of the microscopic anatomy. Several microscopic cellular changes may have occurred a few days after leaves were declared mature on a visible, macroscopic level. Leaves may not have been truly mature until after my initial observation of decrease in force of attachment in the second week. Thus, a better way to assess the difference in temporal age might be to examine the biomechanics and histology of the 5th node every time a new, mature node develops on the plant.

When investigating leaf morphology, both breaking strength and the anatomy of a simple leaf at the 5th node and the first mature compound leaf were examined. While breaking strength and the anatomy did significantly vary between these two morphologies, it would be interesting to see if the properties of a compound leaf differed when compared to a simple leaf at a higher node. A leaf at a higher node would still have the simple morphology, but would be closer in temporal age to the compound leaf.
Examining abscission properties in this manner might better demonstrate, then, if the ease of abscission is influenced more by leaf morphology or leaf age.

Additionally, it would be interesting to examine if abscission properties varied among intact compound leaves and compound leaves missing one or two leaflets. Would the loss of a leaflet resemble a damaged leaf that the plant would likely self-abscise? Or could abscission of some leaflets favor the entire leaf staying on the plant to aid in photosynthesis?

In conclusion, I found that both mechanical and anatomical properties of abscission vary during the life history of *Kalanchoe pinnatum*. Though temporal age did not demonstrate significant changes in mechanical properties beyond the first week, future studies continuing to examine abscission properties of a leaf for an extended period of time might help determine how leaf age affects abscission. Most noticeably, breaking strength varied with phase development. Compound leaflets and side leaflets detached from the plant much more easily than simple leaves, suggesting that a change in leaf morphology from simple to compound may be advantageous to the plant’s asexual reproduction. Furthermore, the differences between direction of force applications suggest that leaf abscission of *K. pinnatum* will more easily occur when shear stress is exerted on the leaf.
Literature Cited


Merlich, F. 1931. Factors Affecting Growth from the Foliar Meristems of *Bryophyllum calycinum.* *Botanical Gazette* 92: 113-140.


Figure 1. Depiction of 4th node through a series of development. Though the 5th node was examined during Summer 2009, A) shows the 4th node when it is almost mature, B) shows the 4th node when it is fully mature, and C) shows the 4th node when it is fully mature and a fully mature 5th node exists above it.
Figure 2. Tensile breaking strength was measured in simple leaves, compound leaves, and compound leaflets by attaching a spring scale to a clamp and attaching it onto the leaf. The spring scale was pulled perpendicularly to the stem until breakage occurred. The stem was supported by two cross beams.
Figure 3. Qualitative description of abscission zones in simple leaves, compound leaves, and compound leaflets. Abscission zones indicated by the area between the white lines. A) There was no distinct abscission zone in a simple leaf, as cell sizes remained similar to those in the petiole. B) Abscission zone in a compound leaf showing distinct layers of uniformly smaller cell sizes compared to adjacent cell sizes in the petiole. C) Abscission zone in a compound leaflet.
Figure 4: Shear breaking strength was measured in compound leaves and compound leaflets by attaching an S-hook to the common rachis (in compound leaf tests) or the petiolule (in leaflet tests). The spring scale was pulled in a downward force parallel to the stem until breakage occurred. The stem and the rachis were supported by two fingers rather than two cross beams to ensure no accidental breakage occurred.
Figure 5. Average breaking strength of simple leaves at the 5th node over a 5 week period. Each point represents the average tensile breaking strength needed to remove the 5th node each week. *To obtain similar sample sizes, the 4th node was used in some cases as it is developmentally similar to the 5th node. Error bars represent the standard deviation of the mean. Tensile breaking strength between weeks were not all equal (df=4, F=3.86, p=.0176). At the 0.05 level, the only significant difference in breaking strength found was between the first and second week (t=3.11, p<0.05).
Figure 6. Average tensile breaking strength for simple leaves, compound leaves, and side leaflets. Each column represents the average tensile breaking strength needed to remove a leaf or leaflet. Average tensile breaking strength between leaves and leaflets were not all equal (df=23, F=53.67, p<0.001). Tensile breaking strength in simple leaves was significantly greater than either compound leaves (t=8.76, p<0.001) or compound side leaflets (t=10.13, p<0.001). Difference in tensile breaking strength between the side leaflet and compound leaf were only marginally significant (df=9, t=1.80, p=0.0860).
Figure 7: Average tensile and shear breaking strength for compound leaves and side leaflets. Tensile breaking strength was significantly higher than shear breaking strength in both compound leaves (df=9, t=4.47, p=0.0016) and compound side leaflets (df=9, t=8.19, p<0.00001). Tensile breaking strength of compound leaves was marginally greater than side leaflets (df=9, t=1.80, p=0.0860) as was shear breaking strength (df=9, t=2.09, p=0.0667).
Table 1. Results of breaking strength measurements in simple leaves at 5th node over time.

<table>
<thead>
<tr>
<th></th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Force (N)</td>
<td>8.1 ± 3.16</td>
<td>7.4 ± 2.22</td>
<td>9.76 ± 3.58</td>
<td>12.12 ± 1.91</td>
<td>12.7 ± 2.81</td>
</tr>
<tr>
<td>Cross-sectional area (m²)</td>
<td>3.25E-06 ± 9.95E07</td>
<td>4.97E-06 ± 3.05E07</td>
<td>5.75E-06 ± 9.88E-7</td>
<td>5.04E-06 ± 8.26E07</td>
<td>4.8E-06 ± 9.2E-07</td>
</tr>
<tr>
<td>Breaking Strength (NM/m²)</td>
<td>2.50 ± 0.78</td>
<td>1.49 ± 0.44</td>
<td>1.74 ± 0.73</td>
<td>2.42 ± 0.22</td>
<td>2.65 ± 0.30</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation of means
Table 2. Results of breaking strength measurements in compound side leaflets and compound leaves.

<table>
<thead>
<tr>
<th>Compound side leaflet</th>
<th>Cross-Sectional Area (m²)</th>
<th>Tensile Force (N)</th>
<th>Tensile Breaking Strength (N/M/m²)</th>
<th>Shear Force (N)</th>
<th>Shear Breaking Strength (N/M/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.78 x 10⁻⁶</td>
<td>3.92</td>
<td>0.58</td>
<td>1.47</td>
<td>0.22</td>
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<td>4.41 x 10⁻⁶</td>
<td>2.81</td>
<td>0.64</td>
<td>1.4</td>
<td>0.32</td>
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<tr>
<td>5.55 x 10⁻⁶</td>
<td>3.31</td>
<td>0.6</td>
<td>1.35</td>
<td>0.24</td>
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<tr>
<td>7.89 x 10⁻⁶</td>
<td>4.98</td>
<td>0.63</td>
<td>1.5</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>7.33 x 10⁻⁶</td>
<td>3.64</td>
<td>0.5</td>
<td>1.29</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>6.75 x 10⁻⁶</td>
<td>3.09</td>
<td>0.46</td>
<td>1.6</td>
<td>0.24</td>
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<tr>
<td>7.69 x 10⁻⁶</td>
<td>4.05</td>
<td>0.53</td>
<td>1.27</td>
<td>0.17</td>
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<tr>
<td>6.65 x 10⁻⁶</td>
<td>3.19</td>
<td>0.48</td>
<td>1.43</td>
<td>0.22</td>
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<tr>
<td>8.03 x 10⁻⁶</td>
<td>4.3</td>
<td>0.54</td>
<td>1.71</td>
<td>0.22</td>
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<tr>
<td>8.41 x 10⁻⁶</td>
<td>3.83</td>
<td>0.46</td>
<td>1.63</td>
<td>0.19</td>
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</tbody>
</table>

Average: 6.95 x 10⁻⁶ ± 1.22  3.71 ± 0.65  0.54 ± 0.07  1.47 ± 0.15  0.22 ± 0.04

<table>
<thead>
<tr>
<th>Compound leaf</th>
<th>Cross-Sectional Area (m²)</th>
<th>Tensile Force (N)</th>
<th>Tensile Breaking Strength (N/M/m²)</th>
<th>Shear Force (N)</th>
<th>Shear Breaking Strength (N/M/m²)</th>
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<tr>
<td>7.96 x 10⁻⁶</td>
<td>5.6</td>
<td>0.7</td>
<td>2.5</td>
<td>0.31</td>
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<tr>
<td>9.58 x 10⁻⁶</td>
<td>9.89</td>
<td>1.03</td>
<td>2.3</td>
<td>0.24</td>
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<tr>
<td>9.42 x 10⁻⁶</td>
<td>8.42</td>
<td>0.89</td>
<td>1.72</td>
<td>0.18</td>
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<td>8.64 x 10⁻⁶</td>
<td>7.52</td>
<td>0.87</td>
<td>2.26</td>
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<tr>
<td>7.25 x 10⁻⁶</td>
<td>4.56</td>
<td>0.63</td>
<td>1.95</td>
<td>0.27</td>
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<tr>
<td>8.68 x 10⁻⁶</td>
<td>6.97</td>
<td>0.8</td>
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<tr>
<td>7.01 x 10⁻⁶</td>
<td>8.65</td>
<td>1.23</td>
<td>2.06</td>
<td>0.29</td>
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<tr>
<td>9.37 x 10⁻⁶</td>
<td>5.44</td>
<td>0.58</td>
<td>2.2</td>
<td>0.23</td>
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<tr>
<td>9.75 x 10⁻⁶</td>
<td>6.28</td>
<td>0.65</td>
<td>2.39</td>
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<tr>
<td>10.43 x 10⁻⁶</td>
<td>6.75</td>
<td>0.65</td>
<td>2.65</td>
<td>0.25</td>
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</tbody>
</table>

Average: 8.81 x 10⁻⁶ ± 1.12  7.01 ± 1.64  0.80 ± 0.21  2.25 ± 0.32  0.26 ± 0.04
<table>
<thead>
<tr>
<th>Table 3a. Simple leaf abscission zone (N=3)</th>
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<tbody>
<tr>
<td><strong>Abscission zone</strong></td>
<td><strong>Petiole</strong></td>
<td></td>
</tr>
<tr>
<td>Total number of cells measured</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Average cell diameter (μm)</td>
<td>31.8</td>
<td>36.3</td>
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<tr>
<td>Standard deviation</td>
<td>7.76</td>
<td>7.06</td>
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<table>
<thead>
<tr>
<th>Table 3b. Compound leaf abscission zone (N=3)</th>
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<tbody>
<tr>
<td><strong>Abscission zone</strong></td>
<td><strong>Petiole</strong></td>
<td></td>
</tr>
<tr>
<td>Total number of cells measured</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Average cell diameter (μm)</td>
<td>15.78</td>
<td>38.7</td>
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<td>Standard deviation</td>
<td>4.56</td>
<td>7.86</td>
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<table>
<thead>
<tr>
<th>Table 3c. Compound side leaflet abscission zone (N=3)</th>
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<tr>
<td><strong>Abscission zone</strong></td>
<td><strong>Petiole</strong></td>
<td></td>
</tr>
<tr>
<td>Total number of cells measured</td>
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<td>30</td>
</tr>
<tr>
<td>Average cell diameter (μm)</td>
<td>14.22</td>
<td>35.70</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>4.15</td>
<td>8.91</td>
</tr>
</tbody>
</table>
Acknowledgments

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