A method for assessing axial and temporal effects of the leaf sheath on the flexural stiffness of the maize stem.

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ABSTRACT

Background: The leaf sheath of many plants has been observed to influence both stiffness of the stem and ultimate strength. The leaf sheath has been implicated in studies of maize “greensnap” (or “brittle-snap”) failure. However, but the influence of the sheath is still not well understood and few methods exist for studying the influence of the sheath. The goal of this study was to develop a method for assessing longitudinal and temporal patterns of sheath influence on flexural stiffness. This metric of flexural stiffness was chosen because it is non-destructive and has been shown to be highly predictive of bending strength.

Results: A three-point bending test method was successfully developed for assessing the influence of the sheath on flexural stiffness. The method relies upon comparisons between pairs of tests at the same location (sheath present vs. absent). The influence of the sheath was statistically significant in all varieties tested. The test method provided insights into the longitudinal and spatial variation of sheath influence: sheath influence appears to be closely related to maturity since both spatial and temporal patterns of influence mirror the sigmoidal maturation patterns previously observed in maize stalks.

Conclusions: The paired nature of this test method increases statistical significance while the non-destructive feature of this test allows for multiple tests along the length of the stalk. This method can be used to provide new insights regarding how the leaf sheath influences stalk flexibility (and therefore strength). Preliminary results indicate that the influence of the sheath changes over the life span of the plant in parallel with maturation patterns. However, further studies will be needed to confirm this hypothesis more broadly and to study additional issues such as heritability and the influence of genotype and environment on sheath influence.

Keywords: maize, greensnap, brittle-snap, flexural, method, leaf sheath
INTRODUCTION

The clasping leaf sheath has been recognized as playing a role in the mechanical response of Poacea species such as *Avena sativa* (oats), *Arundinaria tecta* (switchcane), as well as plants in other families, such as palms (1–3). Recent studies have examined the influence of the leaf sheath on the stiffness and strength on wheat and oat stems (4). The leaf sheath has also been observed to play a role in a phenomenon known as maize greensnap (wind-induced fracture of the stalk during rapid growth) (5,6). Finally, it has been observed that the presence of sheath blight increases the lodging rate in rice (7).

The growth patterns of Poacea plants follow a bottom-up progression in which lower internodes elongate first, while upper internodes elongate last (8). Studies of maize growth dynamics of maize have revealed that both leaf development (including the sheath) and internode elongation follow sigmoidal or “s” shaped curves (9,10).

While the growth patterns of grain plants are well-documented, our overall understanding of leaf/sheath interactions remains relatively sparse. For example, Hill (11) attempted to predict greensnap failure, but concluded that it was not yet possible to predict greensnap susceptibility. A recent study reported that maize stalks are not optimally tapered to resist bending loads (12). However, that study examined stalks in the absence of sheaths.

An deeper understanding of sheath/stalk interactions is needed, but existing bending test methods are inadequate for this purpose. Previous studies that attempted to quantify sheath influence were either destructive (3,4), used hanging weights (2,3), or used relatively complex vibratory methods (1). These prior studies generally provided information only at the level of the entire stalk. Further more, these methods were not designed to assess how the sheath influence varies along the stalk. This is an important limitation, since the literature on stalk/sheath
maturation suggests that the influence of the sheath may vary according to the tissue maturation of the stalk. Whole stalk measurements thus may obscure longitudinal patterns that are important for understanding the influence of the sheath on stalk strength and flexibility.

The development of a robust testing methodology will enable future studies aimed at examining the influence of the leaf sheath in greater detail. For example, it has been hypothesized that further insights into the mechanisms of greensnap could enable metrics for predicting the susceptibility of maize to greensnap failure (11).

The purpose of this study was to develop a technique for measuring the influence of the sheath as a function of axial position along the stalk of large grains such as maize and sorghum. The characteristic of flexural stiffness (also known as flexural rigidity) was chosen for two reasons. First, flexural stiffness is non-destructive. This allows for paired testing between the cases of sheath present vs. absent, which increases statistical power. Second, flexural stiffness has been found to be an excellent predictor of maize stalk strength (13).

**METHODS**

**Maize plants**

Four types of commercial maize were used in this study, including two varieties of field corn, one type of sweet corn, and one type of flint corn. Plants were grown in Spanish Fork, UT in a single location and at a single planting density as these variables were not of particular interest in this study. Plants were grown using the plastic mulch method (14). Plants were irrigated as needed, typically 2-3 times per week. Varieties used in this study included two types of field corn (Vigor root, Silver Queen), one sweet corn (Extra Early), and one flint corn (Fiesta Ornamental).
Specimen Preparation

Stalks were harvested from the field immediately before testing. On each day of testing, a sample of 7 stalks were chosen from each variety. Stalks were selected from random locations within the field, but not from row ends. Pruning shears were used to cut the stalk just below the brace roots. Following collection, all stalks were taken directly to the laboratory for testing.

To prepare for testing, the leaf blades were removed just above the collar using scissors. For testing alignment purposes, stalks marked with a thin black line every 10 cm starting at the basal node using an indelible felt marker.

Flexural Bending Tests

Three-point bending tests were performed using a universal testing machine (Model 3340 Series, Instron, Grove City, PA). Traditionally, three-point bending tests are performed with a load point centered between the two supports. However, this approach was not appropriate in this case because the apical portions of the stalk were sometimes too weak to support the stalk’s own weight. Instead, both supports were placed on one side of the load point, as shown in Figure 1. This configuration is structurally equivalent to a traditional 3-point bending test. This approach allowed the stronger basal region to support the weight of the weaker apical section.

The distances between supports/loads were the same for all tests. Supports were placed 15 cm and 30 cm to the left of the loading anvil. The loading anvil was slowly lowered until the loading anvil made contact with the stalk and induced a slight bending displacement. At this point, the force and displacement were set to zero. The machine then executed three displacement cycles. Each cycle applied a 2 mm displacement to the stalk then returned back to the zero position. This small value for displacement was chosen because it allowed flexural data
to be collected without causing any observable damage to the stalk or sheath (i.e. the
force/deformation path was highly linear). After testing a particular section, the stalk was moved
10 cm to the left, as shown in Figure 1B, and again in Figure 1C. This process was repeated
along the entire length of the stalk.

Stalks were first tested with the leaf sheaths intact. After the entire stalk had been tested,
all the leaf sheaths were removed by scoring the outside of the sheath at the node and carefully
peeling away the sheath. During the sheath removal process, marks were transferred to the stalk.
Each stalk was then tested again at each of the same locations as the initial tests. In this way,
each 30cm section of the stalk was tested with sheaths present and absent and the testing process
was repeated at 10cm intervals along the entire stalk.

Figure 1: An illustration of the test arrangement used in this study. Supports are represented by
gray circles while the red arrow represents the applied force. The gray horizontal arrows
indicate the process of shifting the stalk to the left as the test process progresses.
Flexural Stiffness Calculations

Flexural stiffness (or flexural rigidity) is typically defined in engineering textbooks as $EI$: the product of the Young’s Modulus ($E$) and the area moment of inertia ($I$). Flexural stiffness is most easily obtained by combining force/deformation measurements with the equation for deflection of a beam in three-point bending. Under this approach, the following equation is used to calculate the flexural stiffness:

$$EI = \frac{PL^3}{48\delta}$$

(1)

However, this approach relies upon two assumptions: first, that $E$ is constant within each cross-section of the beam, and second, that both the geometry and the material properties are constant along the length of the beam. Neither of these assumptions are typically true for plant stems. A previous study showed that tissue stiffness variation can be accounted for using a different form for the moment of inertia (15). In this formulation, the material properties are allowed to vary within the cross-section. This produces the material-weighted moment of inertia:

$$I_E = \int E(x, y) y^2 dA$$

(2)

The material-weighted moment of inertia represents the cross-sectional resistance to bending. In other words, $I_E$ captures the flexural stiffness when material properties vary across the cross-section. If $I_E$ is allowed to vary along the length of the stalk, we can write an expression for the deflection using Castigliano’s Theorem:

$$\delta = \int_a^b \frac{\partial}{\partial P} \left( \frac{M(x)}{I_E(x)} \right) d\delta$$

(3)
This equation cannot be evaluated since we do not know the axial variation of $I_E$ (after all, this is the very point of this study!). However, if we assume that $I_E$ is constant within the relatively short test section, we obtain an equation that is very similar to the standard form:

$$\delta = \frac{PL^3}{48I_E}$$  \hspace{1cm} (4)

This can be solved for the flexural stiffness as:

$$I_E = \frac{PL^3}{48\delta}$$  \hspace{1cm} (5)

The use of this expression produces the aggregate flexural stiffness for each tested section. And since we tested each stalk using overlapping sections of 30 cm each, this approach provides an approximation of how the flexural stiffness changes along the length of the stalk.

**Data Analysis**

The data produced by the experiments described above consisted of two sets of flexural stiffness measurements along the length of each stalk: one set representing the flexural stiffness with the sheath present, and a corresponding set of measurements at the same locations, but with the sheath removed. Additional data dimensions included the type of maize and the test date of each data set.
A simple paired t-test was used to assess the overall difference in flexural stiffness between the sheathed and unsheathed conditions. This approach neglects factors such as variety, date, or axial position, but it provides an overall assessment of the influence of the sheath. Paired t-tests were performed by first calculating the percentage difference between sheathed and unsheathed tests according to Equation 6 below. The null hypothesis was that the sheath had no influence on stiffness.

\[
\text{Percent Sheath Influence} = \frac{EI_{\text{with sheath}} - EI_{\text{no sheath}}}{EI_{\text{with sheath}}} \times 100
\]  

The effect of the sheath along the length of the stalk and over time was analyzed using a regression approach. The growth and development of maize internodes and sheaths often follow a sigmoidal (‘S’ shaped) curve (10). Thus, we used a sigmoidal curve as the basis for describing the influence of the leaf sheath on stem stiffness as a function of axial position.

The sigmoidal curve has four key variables, which are illustrated along with the equation in Figure 2. Variable “a” is the upper asymptote, which represents the maximum sheath influence. Variable b is the lower asymptote, which represents the minimum sheath effect. Both a and b have units of percent. Variable c is inversely related to the rate of transition from low to high effect. Lastly, d indicates the point of inflection, the point of maximum rate of change, and the point along the stalk where the sheath has an influence of 50%. Units for variables c and d are in centimeters.
Figure 2: Three sigmoid curves with their associated coefficient values and the sigmoid curve equation.

The collected data was run through a constrained optimization program to calculate the “best fit” values for each variable. Constraints were used to keep each parameter within physically realistic bounds (a and b between 0 and 100, c and d between 0 and stalk height). The fitting process was performed separately for each hybrid/date data set.

RESULTS

Overall Influence of Sheath by Variety

As described in the methods section, the influence of the sheath was quantified as the percent difference in relation to the flexural stiffness measured with the sheath present. The relative influence of the sheath was found to depend upon the axial location along the stalk, the date of measurement, and maize variety. The overall influence of the sheath on each maize type was first examined by ignoring spatial and temporal effects.
For each variety tested, the leaf sheath exhibited a statistically significant effect on the stiffness of the stem. Figure 3 shows the distributions of the relative effect of the leaf sheath for the varieties tested in this study, regardless of other factors. Paired t-tests were performed for each variety, which indicated that the influence of the sheath was highly significant in all varieties (all p-values < 1x10^{-6}). However, as shown in Figure 3, the influence of the sheath varies widely. Much of this variation is due to the remaining experimental factors as well as experimental uncertainty.

**Detecting Spatial Influence of the Sheath**

The spatial influence of the sheath on overall stem stiffness was clearly evident when the
variety and date were held constant. For each date/variety pairing, the influence of the sheath increased with distance from the base of the stalk. This effect was captured by fitting the measured data to the sigmoidal curve described in the methods section. A typical example of this characteristic spatial pattern is shown in Figure 4, in which distance from the base of the stalk increases from left to right and the vertical axis indicates the influence of the sheath.

**Figure 4:** Representative chart showing the sigmoid curve fitted to test results as a function of axial position (additional charts available in the supplementary data that accompanies this paper).

A total of 29 sigmoidal fits of this type were performed (one for each date/variety pair). The $R^2$ values were statistically significant for every sigmoidal fit, thus strongly supporting the concept that leaf sheath influence follows the same sigmoidal pattern that has been used to describe node elongation (9,10). Of these fits, the median $R^2$ value was 0.69, with a mean and standard deviation of 0.65 and 0.15, respectively. These additional charts are available in the supplementary data that accompanies this paper.
Detecting Temporal Influence of the Sheath

Patterns over time are most easily visualized by plotting the values of the sigmoid coefficients as functions of time. Recall from the methods section that parameters a and b indicated the minimum and maximum influence of the sheath, while parameters c and d indicate the rate of increase and location of the inflection point (see Figure 5 for graphical representations of each coefficient). Figure 5 provides plots of each coefficient as a function of time and variety.

As seen in black in the top row of Figure 5, coefficient a (the maximum level of sheath influence) exhibited a statistically significant decrease over time for each variety. In contrast, coefficient b (gray, minimum level of sheath influence) was found to be relatively stable over time.

Coefficient d captures the point of inflection of the sigmoid curve. This coefficient (shown in black in the bottom row of Figure 5), exhibited a statistically significant increase over time for each variety. The temporal patterns of coefficient c were relatively weak. This data indicates that the overall influence of the leaf sheath (the difference between a and b) decreases as time progresses while the point of inflection moves upward along the stalk as time increases.

Coefficients (b and c) did not have a significant pattern across time.
Figure 5: Plots of coefficient values over time for each of the four varieties. Consistent patterns were observed for coefficients $a$ and $d$ (shown in black). In contrast, inconsistent patterns were observed for coefficients $b$ and $c$ (shown in gray).

DISCUSSION

Previous studies have demonstrated that the growth and development of maize follows sigmoidal patterns in both space and time (9,10,16). When comparing across internodes, tissue maturity is highest at the base, and lowest at the apex. The data in this study suggest that the influence of the sheath is correlated with maturity, since both the influence of the sheath and patterns of maturity exhibit the same temporal patterns.

Dissection of the maize internode revealed that immature tissues are soft, flexible, and have relatively low strength. In contrast, more mature tissues are increasingly hard, inflexible, and have apparently higher strength. These observations, when combined with the data collected in this study suggest that the leaf sheath provides critical structural support for these immature tissues. Thus, as the tissues mature, the influence of the sheath decreases.
At the level of the entire stem, the patterns observed in this study are illustrated in Figure 6. This Figure shows the temporal pattern of sheath influence as a function of time. In this chart, arrows indicate the passage of time, with sigmoidal curves of sheath influence depicted at four equally spaced time points. The Figure shows that the point of inflection moves upward along the stalk as the upper asymptote simultaneously moves downward. This observation has implications for future studies on the growth and development of grain species, and for studies focused on greensnap maize failure (11).

Figure 6: Illustration of the typical progression of coefficients a and d, and the resulting progression of sigmoid curves.

Practical Considerations

As with all test methods, this method has advantages and disadvantages. Advantages of this test method include the following: (1) minimal sample preparation; (2) the test does not induce structural damage of the stalk; (3) the test supports are fixed throughout the test, which results in a significant time savings over methods which require adjustment of the supports for each test (e.g.,17,18); (4) longitudinal resolution can be adjusted by broadening or shortening the
distance between the applied load and the left support; (5) the paired nature of the test increases statistical significance.

Disadvantages of this test method include the following; (1) the removal of the sheath can be somewhat time-consuming; (2) the attainment of high spatial resolution along the stalk increases the number of tests that must be performed and may cause a degradation in data quality as a shorter span increases the tendency for transverse compression of the stalk (15,17,18); (3) the test requires a non-standard three-point bending test configuration.

Limitations and Future Research

The purpose of this study was methodological development, not to specifically quantify the influence of sheath on any particular variety or at any particular period of growth. Hence some factors that may influence sheath/stalk interaction were not quantified or varied. For example, field replicates and multiple growing environments were not used in this study and time was quantified using regular dates rather than by using growth-degree-units or phyllochrons. This preliminary study provides a methodology and framework for future studies that will make use of more sophisticated methods of quantifying and modeling the influence of time and temperature on the sheath influence.

Dissection of maize internode and observed differences between immature and mature tissues, including tissue stiffness, flexibility, and strength were only qualitative in nature (and hence were not included in the results section). However, these observations provide valuable insights for future researchers. This study did not seek to quantify the mechanical differences between immature and mature tissues. These types of measurements will allow for a more complete description of the phenomena observed, and allow a more comprehensive description
of the tissue maturation process. Unfortunately the authors are unaware of any formal methods for making such measurements. Furthermore, the authors’ prior experience performing similar measurements suggests that significant methodological development will need to be performed in order to enable such measurements. Thus, future research in this area will need to focus first on the development of testing methodologies, as well as the application of these methodologies to the study of maize tissue development.

CONCLUSION

This paper provides a description of a testing methodology that can be used to quantify the influence of the leaf sheath along two important dimensions: along the length of the stem, and across time. The results of these preliminary tests suggest that the leaf sheath of maize plays an important structural role by increasing stem stiffness (and possibly increases stalk strength). The influence of the sheath was observed to decrease over time, both locally at the individual internodes, and globally, across the entire length of the stem. The data collected in this study indicated that the leaf sheath significantly increased stem stiffness in each variety tested. Future tests of this type will be needed to further elucidate the nature and more specific effects of the leaf sheath on issues such as greensnap failure and tissue maturation.
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Authors' contributions: JH performed the statistical analysis and aided in testing, SW and NH aided in maize cultivation and performed the experiments. CS contributed to the design of the test procedure as well as aiding with initial tests, DC contributed to test design, field work, and conceptual design of the statistical analysis.

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