An Improved Method for Accurate Phenotyping of Corn Stalk Strength

Daniel Robertson, Simeon Smith, Brian Gardunia, Douglas Cook*

ABSTRACT
Weak stems or stalks in grass crop species increase the likelihood of stalk failure, thereby reducing yield. Three-point bending tests are often employed in selective breeding studies to characterize stalk strength. However, it is hypothesized that the loading setup used during three-point bending experiments may significantly alter test results. To investigate this hypothesis, two different loading configurations were employed in conducting three-point bending experiments of corn (Zea mays L.) stalks. In the first configuration, stalks were loaded and supported at nodes. In the second configuration, stalks were loaded and supported at internodal segments. Significantly higher bending moments were experienced at internodal segments during the node-loaded configuration than was required to fail the same segment during internode-loaded tests. This is because the loading anvil significantly deforms the stalk’s cross section when it is placed on an internodal segment, thereby inducing premature failure. In addition, internode-loaded tests were observed to produce unnatural failure patterns, while node-loaded tests demonstrated natural variability in failure location. While transverse deformation of the stalk cross section cannot be eliminated in three-point bending tests, its effects can be mitigated by placing the loading anvil at nodal locations, which are much stiffer than internode regions. Maximizing the span length of bending tests likewise reduces transverse deformation of stalk cross sections. These results are relevant to selective breeding studies designed to produce lodging resistant crop hybrids.

STEM OR STALK STRENGTH is particularly important for grass crop species like wheat (Triticum spp.), rice (Oryza sativa L.), and corn because weak stalks often reduce yields for farmers. Stalk strength is a genetically determined trait that can be affected by both abiotic and biotic factors, including disease pressure, climate, and soil features. These factors reduce heritability and make it difficult to characterize stalk failure (lodging), even when collecting data across multiple years and across varied environments (Cloninger et al., 1970; Dodd, 1980). Various mechanical tests have been developed to measure stalk strength. Crush tests were developed and applied to excised maize stalk segments (Thompson, 1964; Zuber and Grogan, 1961) to select stronger stalks. Rind penetrometers have been used for recurrent selection and quantitative trait locus (QTL) mapping (Peiffer et al., 2013) of maize, and three-point bending has been used for QTL mapping and corn stalk characterization (Hu et al., 2013). Recent studies in other grass species have used similar approaches (Jin et al., 2009; Kokubo et al., 1991; Li et al., 2003; O’Dogherty et al., 1995). A standard three-point bending test setup has not been established in the plant biomechanics field. However, the most common technique is to place the loading anvil at the center of an internodal segment while supporting the stalk at the same internode or

D. Robertson, S. Smith, and D. Cook, Dep. of Mechanical Engineering, New York Univ.–Abu Dhabi, PO Box 129188, Abu Dhabi, United Arab Emirates; and B. Gardunia, Monsanto Corporation, 1551 Highway 210, Huxley, IA. Received 28 Nov. 2013. *Corresponding author (dc125@nyu.edu).

Abbreviations: QTL, quantitative trait loci.

Published in Crop Sci. 54:2038–2044 (2014).
doi: 10.2135/cropsci2013.11.0794

© Crop Science Society of America | 5585 Guilford Rd., Madison, WI 53711 USA
This is an open access article distributed under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
at adjacent nodes. This paper focuses on the use of three-point bending tests for measuring the structural properties of corn. Specifically, we address how three-point bending tests may be modified to provide more accurate phenotyping, thereby increasing heritability of stalk strength and lodging resistance.

Three-point bending flexural tests (Fig. 1) have been used in the field of plant biomechanics for more than 100 yr (Tiemann, 1906). Recently, bending tests have been used to investigate crop lodging (Berry et al., 2000, 2006, 2007; Verma et al., 2005), correlations between density and strength (Niklas, 1993), failure modes (Liu et al., 2004), and molecular mechanisms (Li et al., 2003), among others. Three-point bending is the predominant type of flexural test, and it provides several advantages as compared with other testing methods, including ease of specimen preparation, ease of test setup, and minimal post processing of data. Material properties such as modulus of elasticity, flexural stiffness, and even fracture toughness can be estimated using data from three-point bending experiments. However, the method is not without disadvantages. In all three-point bending tests, a concentrated transverse load is applied at or near the midpoint of the test specimen. For solid objects such as beams or solid stems, this poses no immediate complications. However, for crop species, a transverse load could potentially crush and/or crease the stem, causing the stalk to break (fail) prematurely. In our laboratory, we noticed that loading corn stalks at the internode caused deformation patterns, failure modes, and failure locations that were substantially different from in-field observations of lodged corn stalks. The authors’ own observations concur with the published literature that stalks typically fail just above a node (Niklas, 1992, 1998).

The purpose of this study was to investigate the effect of test configurations on the results of three-point bending tests involving corn stalks. We hypothesized that loading configuration does affect three-point bending test results, with lower maximum loads recorded when the load is placed at the internode as compared with the node. We further hypothesized that the application of the transverse load at the node reduces artifactual results and produces failure patterns more consistent with in-field observations. This study was performed to test these hypotheses and to develop a testing protocol that provides reliable, objective measures of corn stalk strength.

**METHODS**

Stalk samples were drawn from four commercial varieties of dent corn and at four different planting densities. Eight sets of stalks were sampled, with five stalks in each set, for a total of 40 stalks (hybrids A and B at planting densities of 36 thousand and 48 thousand plants per acre, hybrid C at planting densities of 30 thousand and 48 thousand plants per acre, and hybrid D at 30 thousand and 42 thousand plants per acre). All stalks were harvested in Greenville, IA immediately before harvest. Stalks were cut just above ground level; the ear was removed along with the portion of the stalk above the ear node. To prevent spoilage, stalks were placed in a forced air dryer to reduce stalk moisture level to between 10% and 13%. All stalks were checked for disease and pest damage. Only stalks exhibiting no visible evidence of damage were included in the study.
Flexural Tests
To test the hypothesis that loading location influences failure load levels, three-point bending tests were performed with two different loading configurations: (i) the load applied at the center of the internode region and (ii) the load applied immediately below the node line.

Tests were conducted using an Instron 5965 test frame with a 500-N load cell. All stalks were oriented such that the loading direction was parallel to the minor axis of the stalk cross section. During testing, the load anvil was displaced at a rate of 10 cm min\(^{-1}\). Load and displacement data were recorded every 100 ms, just until failure of the stalk was detected. Failure was defined as the point on the force-displacement curve where load began to decrease with increasing deflection. This occurs due to irreversible damage to the structural integrity of the stalk (e.g., cell walls crushing or buckling, stalk breakage).

To facilitate comparison and reduce experimental variation, each stalk was tested in both three-point bending configurations. Node loading tests were performed first. The load was applied at a central node, with the stalk supported at the second nodes above and below the loaded node. This loading configuration consistently produced failure near the loaded node, with failure always restricted to one side of the node (i.e., failure did not propagate through the node). The non-damaged, adjacent internode was then used for subsequent internode testing. A span length of 100 mm was used for all internode tests.

Strength Comparisons
Bending failure is governed by the bending moment, which depends on two factors: the applied force itself and the span between the force and supports (see Fig. 1). When subjected to three-point bending, internal forces (called bending moments) act to cause bending at each cross section along the span of the structure. The bending moment at any given point along the length of the structure can be found using Eq. [1].

\[
M(x) = \begin{cases} 
  P x & | 0 \leq x \leq L / 2 \\
  P(L - x) & | L / 2 \leq x \leq L 
\end{cases} \tag{1}
\]

Bending tests were used to make two types of comparisons. First, comparisons between node-loaded and internode-loaded sections were made by direct comparison of the bending moment at failure for both tests. Although this approach provides a comparison of the moments required to cause failure, differences in the cross-sectional shape, size, rind thickness, etc. between the two tests could potentially affect the results. A more relevant and direct comparison is possible by calculating the internal bending moment that acted at the internode region during the node-loading experiments. For example, using Eq. [1] and the loading configuration of Fig. 1, we can determine that the bending moment, \(M\), at the point \(x = L/4\) is \(M = PL/4\).

Therefore, the second method of comparison was to calculate the maximum bending moment applied at the internode during the node-loaded test. This quantity was then compared with the maximum bending moment applied during internode-loaded experiments. This approach accounts for all cross-sectional and size factors, providing a direct comparison by which the effect of internode loading can be evaluated.

Statistical Tests
A statistical comparison of the means from each type of test was performed using a two-sample \(t\) test. The two-tail \(p\)-value was calculated from the resulting student \(t\) test statistic. For comparisons between bending moment applied at the internode during node-loaded and internode-loaded cases, a two-sided paired difference test was used to determine if there were statistical differences in stalk strength under node-loaded or internode-loaded cases.

Transverse Compression Tests
In bending experiments, stresses, stiffness, and failure are all closely related to cross-sectional shape. The concentrated load applied during three-point bending experiments has the potential to deform the cross-sectional shape of the corn stalk, thus reducing bending stiffness and possibly contributing to premature failure. To assess the influence of the transverse load on stalk shape, the cross-sectional stiffness of the stalk was measured at various points along the length of the stalk.

Cross-sectional stiffness was measured using the same machine and loading fixtures as described above. Intact stalks were compressed by opposing forces, with the load applied directly above the support. The upper anvil was displaced downward, causing a compressional force. To measure stiffness without damaging the stalk, all tests were performed with a maximum deformation of 0.2 mm and a loading rate of 2 mm min\(^{-1}\). Cross-sectional stiffness was determined as the average slope of the resulting force-deformation curve, and this test was repeated at intervals of 5 mm along the length of the stalk.

RESULTS
All stalks exhibited remarkably similar behavior under each of the experiments described above. Consequently, the data below represent aggregate results based on the combination of all stalks into a single sample (\(n = 40\)) consisting of four different commercial hybrids grown at four different planting densities.

Differences between node-loaded and internode-loaded tests are depicted in Fig. 2a and 2b using box plots of the bending moment at failure for both the node and internode tests. Results show that stalks that were loaded at the internodes failed at significantly lower moments than when loaded at the nodes (\(p < 0.001\)). A second, more direct comparison of moments is made in Fig. 2b. As discussed above, this comparison is based on the maximum bending moments applied to the center of the same internode during the node-loaded and internode-loaded tests. Figure 2b shows that a much higher bending moment was experienced at the internode during the node-loaded test than was required to fail the same
section during the internode-loaded test. On average, when loaded at the internode, stalks failed at a bending moment that was 54% lower than the bending moment applied at the same point during the node-loaded tests. Paired \( t \) tests were performed for each set of five stalks and for the entire set of 40 stalks. For individual sets of five stalks, all were statistically significant, with the largest \( p \)-value obtained being \( p = 0.015 \). For the combined set of 40 stalks, the result was highly significant, \( p = 1.0 \times 10^{-13} \).

It is important to note that failure did not occur at the internode during the node-loaded test. In other words, every internode in this study withstood a higher bending moment during the node-loading test than the moment that caused failure during the internode test. Because node-loaded data of Fig. 2b represents unfailed stalks, while the internode-loaded data represents failed stalks, the reported 54% reduction in failure moment should be interpreted as a minimum estimate for the difference induced by loading at the internode.

All stalks exhibited similar cross-sectional stiffness patterns in which the internode region was less stiff than nodal regions. Transverse compressive stiffness versus distance along the stalk is given for a representative corn stalk in Fig. 3. An X-ray image of the same stalk is shown at the bottom of the figure for anatomical reference. The distinct, periodic peaks in stiffness observed in Fig. 3 are a consequence of the morphological and anatomical structure of corn stalk. In particular, peaks in stiffness coincide with each node, which is shown as the darker tissue in the X-ray image. Not only does the stalk diameter increase in nodal regions but rind thickness and tissue density increase as well (see X-ray image). These differences in nodal tissues substantially increase the node’s ability to support transverse loads. In fact, comparison of the stiffness values calculated at the node and the center of each internode indicate that nodes are more than twice as stiff as internodes (one-tail \( t \) test, \( p < 0.001 \)). As such, nodes are a prime location to place the loading anvil of three-point bending experiments because they are far more resistant to localized cross-sectional deformation.

Figure 4 depicts the failure locations of internode-loaded stalks and node-loaded stalks. Representative pictures of the type of failures are displayed in Fig. 5. We found that all internode-loaded stalks failed in the same location and in the same manner: a single creased line located at the point where the loading anvil contacted the stalk. This pattern of failure is not similar to observations of in-field failures. Stalks failed under natural loading conditions typically display variation in both type and location of failure. Results from node-loaded three-point bending tests agreed well with in-field failure observations: they demonstrated variability in location, type, and path of tissue failure across the stalk (see Fig. 5). For node-loaded stalks, failure occurred away from the loading anvil and just above the node, with slight variations in the exact location of failure. In addition, failure crease patterns of node-loaded test specimens were unique for each stalk. These results suggest natural variation in the location and type of failure when loaded at the node.
Figure 3. Transverse compressive stiffness measured at 0.5-mm intervals along maize stalk. An X-ray image of the same maize stalk is shown for physiological reference. Distinct peaks in the stiffness are seen to coincide with the nodal regions (dark bands in X-ray image).

Figure 4. Distribution of normalized failure locations for node (green) and internode (red) loaded three-point bending tests of corn stalk. The X-ray image references location along the stalk, with green and red lines showing load points for node and internode tests, respectively.

Figure 5. Images of general failure types and locations for internode (top) and node (bottom) loaded three-point bending tests of corn stalk.
Figure 6 displays photographs of node-loaded and internode-loaded corn samples immediately before failure. Significant transverse deformation was apparent at and around the loading anvil before failure in all internode-loaded samples, as shown in Fig. 6A. During internode-loaded tests, cross-sectional deformation at the load location occurred almost immediately on contact of the loading anvil with the stalk. In contrast, node-loaded stalks (Fig. 6B) exhibited obvious bending deformation, but no visible transverse compression of the stalk.

**Discussion**

**Deformation and Failure of Thin-Walled Stalks**

Under three-point bending, a transverse load is applied as a means of imposing a bending moment on the specimen. This transverse load has two effects on the stalk, regardless of loading location. The first is intentional: to cause a bending moment, which acts to bend the stalk (Fig. 1). The second is an unintentional consequence of the first: the load imposes transverse compression on the stalk (see Fig. 3 and 6). We observed that the manner in which bending and transverse compression affects the stalk depends on the point of load application. The following two paragraphs describe these interactions.

When a load is placed at the internode region, transverse deformation (Fig. 6A) is significant, and bending deformation is often minimal. This is due to the relatively low transverse stiffness of the internode region (Fig. 3). Deformation of the cross-section reduces the stalk’s ability to resist bending stresses and causes it to be especially susceptible to buckling failure. Eventually, the concentrated load of the anvil, combined with the relatively low transverse stiffness of the stalk, cause creasing of the stalk at the point of loading. A nearly straight crease propagates along the stalk rind, leading to the failure patterns shown in the top row of Fig. 5 and the red data of Fig. 4. For internode-loading, compressional stresses appear to be the dominant cause of failure. The lack of variation in failure pattern and location indicate that such failure is a result of interactions between the stalk and the test instrument. Under node loading, the internode region supported an average bending moment of approximately 10 Nm without failure. In contrast, the same region failed under an average bending moment of just 4 Nm when the load was placed at the internode.

Transverse and bending deformation also both occur when the load is placed at the node. However, the high transverse stiffness at the node (Fig. 3) results in negligible transverse compression at the node (Fig. 6B). As the load increases, bending deformation therefore dominates, and bending stresses eventually cause failure near (but not at) the loading point. Failures of node-loaded stalks vary in both failure pattern and location (Fig. 4 and 5), suggesting that these failures are determined by local irregularities and/or weaknesses in the stalk.

**The Influence of Span Length**

Span length affects the force levels of three-point bending tests. There are a number of load-span combinations that can be used to achieve any bending moment. As the span increases, the force required to achieve a prescribed moment decreases (and vice versa), as shown in Fig. 1. Even though the node is stiffer and stronger than the internode under transverse compression, it is not indestructible. During other experiments in our laboratory, node regions have been crushed in transverse compression when the span length is quite short. Thus, in addition to the loading point, the span length of three-point bending tests should be considered when designing three-point bending tests. In general, we suggest maximizing the span length to minimize the transverse load.

**Limitations**

While this study was based on maize, it is anticipated that these results can also be extended to species having a similar structure, such as reeds and other grains. The stalks tested in this study were all collected immediately before harvest and were dried to prevent spoilage. While the drying process might have affected the precise values of our tests, we do not believe that the conclusions would be affected because the change in moisture level between harvest and drying is relatively small (~5–10%).

---

Figure 6. Images of corn stalk captured immediately before failure, illustrating differences in deformation patterns. (A) Internode loading. (B) Node loading. A horizontal line is provided as a reference in each image.
In addition, the drying process does not affect the overall structure of the stalk, which is characterized by stiff nodes and internode regions, which are less stiff. On the other hand, we do not feel that our results can necessarily be extended to the measurement of green stalks. While such stalks might be expected to be stiffer at the nodes, turgor pressure might act in such a way as to make nodes and internode regions equally resistant to transverse loading. Additional study is probably warranted on this topic.

One important factor that we expect to affect the relevance of these results to other species is the ratio of rind thickness to stalk diameter. As this ratio varies, the effects of transverse compression can be expected to change; stalks having a very thick rind (relative to the diameter) may be expected to be less susceptible to the internodal failure patterns shown above. The relationship between rind thickness and transverse compression of stalks should also be explored in future studies.

CONCLUSIONS

This study has provided insight into the failure mechanisms of corn stalks under three-point bending. We have shown that transverse compressional stiffness varies substantially along such stalks and that the results of three-point bending tests can be influenced by the choice of loading location. Transverse loads were shown to cause substantial deformation of the stem cross section during internode-loaded tests. This deformation causes premature failure, resulting in (i) failure patterns that are unlike those observed in the field and (ii) measured moments that are artificially low.

While transverse deformation cannot be eliminated in three-point bending tests, its effects can be mitigated in two ways. First, we recommend that loads be placed at nodal locations, which are much less susceptible to cross-sectional compression than the internode regions. Second, we recommend maximizing span length of bending tests as a means of reducing transverse loads. Consideration of these factors can be used by researchers to design three-point bending tests that provide accurate, reliable measurements of corn stalk stiffness and strength. We observed that node-loaded stalks’ failure patterns more closely resemble naturally occurring failure patterns and locations than other commonly used test methods (i.e., crush tests, rind penetrometry, four-point bending, etc.). As such, node-loaded three-point bending tests are likely to provide improved phenotyping accuracy in selective breeding studies seeking to increase stalk strength and lodging resistance.

Acknowledgments

Stalk samples were graciously provided by Monsanto Corp.

References


Tiemann, H.D. 1906. Effect of moisture upon the strength and stiffness of wood. USDA Forest Service, Washington, DC.
