Accepted Manuscript
Version of Record at: https://doi.org/10.1093/jxb/erae465

# A Tool to Measure Maize Root System Stiffness That Enables a Comprehensive Understanding of Plant Mechanics and Lodging

*Running Title: Root system stiffness relates to root lodging susceptibility*

Authors
Ashley N. Hostetler[1*], Jonathan W. Reneau[1*], Joseph Cristiano[1,2], Teclemariam Weldekidan[1], Taran A. Kermani[1,3], Therese T. Kim[4], and Erin E. Sparks[1]

*These authors contributed equally to this project.

Affiliations:
[1] Department of Plant and Soil Sciences and the Delaware Biotechnology Institute, University of Delaware, Newark, DE, USA
[2] Department of Electrical and Computer Engineering, University of Delaware, Newark, DE, USA
[3] The Charter School of Wilmington, Wilmington, DE, USA
[4] Newark Charter School, Newark, DE, USA

Contact information for corresponding author: Erin E. Sparks esparks@udel.edu

Author email addresses:
- ahende@udel.edu
- jreneau@udel.edu
- jcristia@udel.edu
- tecle@udel.edu
- kermani@udel.edu
- therkim24@ncs.charter.k12.de.us
- esparks@udel.edu

Date of Initial Submission: Tuesday, June 25, 2024

Number of Tables & Figures:
Tables: 0
Figures: 7

Word Count: 4,688

Number of Supplementary Tables & Figures:
Supplementary Tables: 12
Supplementary Figures: 5
Supplementary Videos: 1

Keywords: anchorage, strength, biomechanics, failure, plant density, maize

Abbreviations:

SMURF (Sorghum and Maize Under Rotational Force); dap (days after planting); GDD (Growing Degree Day)

## Highlight

We report a tool to quantify root system stiffness in cereal crops and show that it can be used to evaluate root lodging susceptibility in maize.


## Abstract

Plant mechanical failure, known as lodging, has detrimental impacts on the quality and quantity of maize yields. Failure can occur at stalks (stalk lodging) or at roots (root lodging). While previous research has focused on proxy measures for stalk stiffness, stalk strength, and root strength, there is a need to quantify the root system stiffness, which quantifies the force-displacement relationship. Here we report a tool to quantify the root system stiffness of maize hybrids grown in different conditions. Results show that maize hybrids with a higher root system stiffness have a greater susceptibility to root lodging. This result is consistent with expected mechanical behavior, since higher root system stiffness values mean that the plant reaches the failure strength at lower displacements compared to a plant with lower root system stiffness. Collectively, this study describes the first tool to measure root system stiffness and enables a comprehensive understanding of the integrated plant mechanics and lodging.


## Introduction

In agriculture, a failure of plants to remain upright is called lodging and has devastating impacts on crop yield. Lodging is defined as a stalk displacement from vertical, with a delineation between stalk and root lodging based on the location of failure (Berry *et al.*, 2004; Rajkumara, 2008). For stalk lodging, failure is binary – the stalk is either broken/buckled or it is not (Robertson et al. 2015; Erndwein et al. 2020). For root lodging, failure is a spectrum that can be defined by the degree of displacement (Wilson, 1930; Hostetler *et al.*, 2022; Zheng *et al.*, 2023). The spectrum varies from plants that have a few broken roots to plants that are completely uprooted with the plant laying on the ground (Erndwein *et al.*, 2020; Hostetler *et al.*, 2022). Despite the spectrum of root lodging, it is often studied in the context of complete failure and reported as a single force value called strength (Zuber *et al.*, 1971; Fincher *et al.*, 1985; Kamara *et al.*, 2003; Liu *et al.*, 2011). Strength is a measurement of plant mechanics.

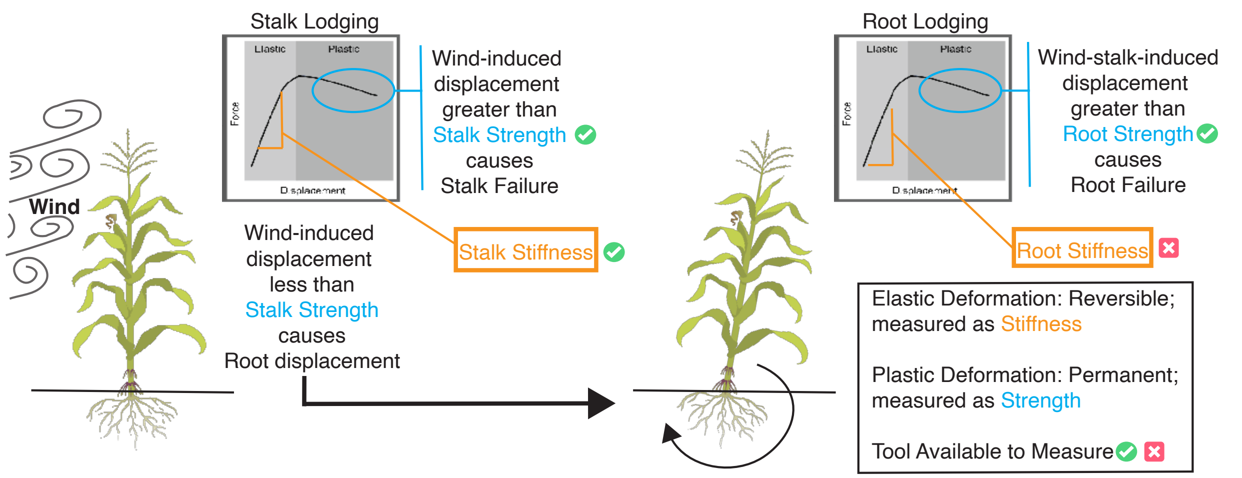


**Figure 1. Schematic of the plant mechanical basis of lodging.** When wind interacts with a plant, it is perceived by the stalk and induces stalk displacement. If the wind-induced displacement exceeds the stalk strength, the stalk will fail, called stalk lodging. For wind-induced displacements that are less than the stalk strength, the displacement translates into root displacement. If the wind-stalk-induced root displacement exceeds the root strength, the root will fail, called root lodging. For both stalk and root, strength has been reported as the force to complete failure (e.g. breaking or uproot), and stiffness represents the rate at which the displacement reaches that force. We argue that any plastic deformation indicates structural failure and will contribute to lodging. There are currently tools available to measure stalk stiffness, stalk strength, and root strength, but not root stiffness.

From a mechanical perspective, plants are composite materials that can be simplified as linear-elastic and have two mechanical behaviors (elastic and plastic)(Niklas and Spatz 2012). Under low displacements, plants exhibit elastic behavior, which results in plant deformation without permanent changes (Niklas and Spatz 2012). At higher displacements plants exhibit plastic behavior, which introduces irreversible and permanent changes (Niklas and Spatz 2012). As highlighted above, for root lodging these permanent changes can span the spectrum from a few broken roots to complete uprooting (Erndwein *et al.*, 2020; Hostetler *et al.*, 2022).

Quantifying plant mechanics and selecting root lodging resistant genotypes requires an understanding of the integrated plant mechanical system, which has been outlined in **Figure 1**. During a storm, the wind interacts directly with the stalk. The stalk mechanics then determine how much the stalk bends (stalk stiffness) before breaking (stalk strength) (Obayes *et al.*, 2022; Stubbs *et al.*, 2023). Simultaneously, the stalk transmits these forces to induce displacement of the root system. While it has been shown that stalk mechanical behavior is directly related to the support of the root system (Reneau *et al.*, 2020; Hostetler *et al.*, 2022; Obayes *et al.*, 2022), it is unclear how variable stalk mechanics relates to root system displacement. Increasing plant height (i.e., changing stalk mechanics) by increasing planting density results in a greater susceptibility to root lodging (Liu *et al.*, 2012). This suggests that varying stalk mechanics will alter root system displacement, but a holistic assessment of plant mechanics has not been reported.

Like stalk mechanics, root mechanics determine how much the root system bends (root stiffness) before breaking (root strength). Understanding plant mechanical failure (lodging) requires the ability to measure all aspects of plant mechanics. Currently, there are tools that measure stalk stiffness, stalk strength, and root strength (Erndwein *et al.*, 2020), but not root system stiffness.

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In this study, we report a tool designed to fill the gap in assessing plant mechanics that measures the root system stiffness. Using the **S**orghum and **M**aize **U**nder **R**otational **F**orce (SMURF) device in maize, we demonstrate that hybrid plants with higher root system stiffness have greater root lodging susceptibility. We further show that when plants are grown at higher planting densities, the root system stiffness is reduced, which is consistent with a reduction in root system architecture. Regardless of root system strength, a higher root system stiffness will reach failure at lower displacements. These results validate the importance of measuring root system stiffness to quantify whole plant mechanics.

## Materials and Methods

### Plant Material

Experiments were conducted at a Newark, DE USA field site (39°40′ N and 75°45′ W) across three years: 2020, 2021, and 2022. Seeds were planted on 06/26/2020, 05/20/2021, and 05/23/2022. All experimental plots, across all years, were surrounded by at least 1-row of border plants and were part of our larger 1200+ plot field site.

*Device Repeatability Experiments*

To assess testing parameters and potential constraints of the SMURF device, two experiments were conducted. In the first experiment, testing the same plant more than once was assessed. For this experiment, maize inbred line, CML258, was grown in 2021 at a density of 18 plants per 3.66 m single row plots with 0.762 m spacing between rows (6.4 plants/m$^2$). In the second experiment, time of testing was assessed. For this experiment, one maize inbred line (CML258) was grown in 2021 and 2022, and two maize inbred lines (B73 and W22) were grown in 2021 at the same density.

*Assessment of Root System Stiffness Among Maize Hybrids*

Maize hybrids that were previously shown to vary for root lodging susceptibility (Tirado *et al.*, 2021) were assessed in 2020, 2021, and 2022. In 2020 and 2021, the 11 hybrids were grown at a density of 10 plants per 3.66 m single row plots with 0.762 m spacing between rows (3.6 plants/m$^2$), and in 2022 the 11 hybrids were grown at a density of 18 plants per 3.66 m single row plots with 0.762 m spacing between rows (6.4 plants/m$^2$). Across all three years, all hybrids were grown in 2-4 replicate plots. The 11 hybrids used in this experiment were DK3IIH6 x LH198, LH145 x DK3IIH6, LH198 x PHN46, LH82 x DK3IIH6, LH82 x LH145, LH82 x PHK76, LH82 x PHP02, PHB47 x LH198, PHB47 x PHP02, PHK56 x PHK76, and PHK76 x PHP02.

*Assessment of Root System Stiffness and Plant Density*

Previous research has shown a relationship between plant density and root system size (Liu *et al.*, 2012). To assess this relationship and the functional impact, two maize hybrids were evaluated in 2022 at four plant densities. Specifically, LH82 x DK3IIH6 and LH82 x PHP02 were planted in two replicate 4-row plots per density. Densities were as follows: 6 plants per row (2.1 plants/m$^2$), 12 plants per row (4.3 plants/m$^2$), 18 plants per row (6.4 plants/m$^2$), and 24 plants per row (8.6 plants/m$^2$). Each row is 3.66 m row with 0.762 m spacing between rows.

Field Management

Our field site has overhead linear irrigation and is treated with pre-emergence herbicides, post-emergence herbicide, soil insecticide, and fertilizers. Specifically, Lexar (at 8.18 L $ha^{-1}$) and Simazine (at 2.81 L $ha^{-1}$) are used as pre-emergence herbicides, and Accent (at 0.05 L $ha^{-1}$) is used as a post-emergence herbicide. At the time of planting, COUNTER 20 G was used as a soil insecticide (at 6.16 kg $ha^{-1}$), and ammonium sulfate 21-0-0 (at 100.88 kg $ha^{-1}$) was used as a starter fertilizer. Approximately one month after planting (when plants are knee high), 30% urea ammonium nitrate was applied (at 374.16 L $ha^{-1}$). Weather data can be found at the Delaware Environmental Observing System (DEOS; www.deos.udel.edu, select the Newark, DE-Ag Farm station).

Assessment of Relative Root Lodging Susceptibility of Hybrids

During the 2020 field season, at 39 days after planting (dap), Tropical Storm Isaias caused root lodging at our field site. Winds were from the West and Northwest and averaged 10.6 m/s as measured from the DEOS station on our farm at a height of 2 m. The day after the storm (08/05/2020), root lodging was manually quantified for each plot. Plants were considered root lodged if their roots were pulled out of the ground or broken. Hybrids were categorized as having low, moderate, or severe levels of root lodging according to their response to Tropical Storm Isaias. Hybrids were classified as low when root lodging from both plots ( $\frac{root\ lodged\ plants}{total\ plants} \times 100$) was more than 1 standard deviation below the population mean for root lodging and were considered severe when root lodging from both plots was more than 1 standard deviation above the population mean for root lodging (Hostetler *et al.*, 2022). Consistent with our previous report on root lodging at the Newark, DE field site (Hostetler *et al.*, 2022), we again observed no relationship for root lodging between the direction of the wind and plot position (**Supplemental Figure S1A**).

Aboveground Phenotyping of Stalk Characteristics Across Planting Densities

In 2022, aboveground plant phenotype data was collected manually at 70 dap for the two hybrids grown at four planting densities. Plant height was measured at approximately the R5 stage after the ear was fully developed and was defined as the distance from the soil to the top of the tassel peduncle. The major and minor stalk diameter were quantified at 54 cm (approximately 25% of the plant height) from the ground with calipers.

Quantification of Root System Stiffness

*Device Design & Operation*

SMURF (**S**orghum and **M**aize **U**nder **R**otational **F**orce) was designed to non-destructively measure the root system stiffness of large grain root systems within the field context. The device consists of a physical body, electronics, powertrain, sensors, and software (**Supplemental Figure 2A**). The physical body is composed of five components: 1) a 3D-printed case housing the electronics and powertrain, 2) a battery pack, 3) a set of arms with straps to attach the device to the plant and limit stalk bending, 4) a sensor housing case for the load cell, and 5) a rotational foot for device grounding. The internal electronics are run via an Arduino microcontroller connecting a motor driver, load cell amplifier, and Bluetooth module (**Supplemental Figure 2B**). The powertrain consists of a brushed DC motor, lead screw, and battery. The sensor is a 10 kg load cell. The device is controlled by a custom-built iPad application that communicates via the Bluetooth module.

For acquiring data on the entire root system (underground roots and brace roots that enter the soil), the SMURF was attached directly above the highest node of brace roots that entered the soil. The straps used to attach the device to the plant were tightly secured to prevent slippage along the stalk. After attachment, the device balanced its own weight and tared the load cell. For testing, the lead screw extended 15.8 mm (**Supplemental Video 1**). Displacement (mm) and force (kg) were recorded from the device.

*Data Processing*

The SMURF device records force-displacement data in kg and mm. These data were then converted into newtons (N) and meters (m). The slope of the force-displacement curve was then extracted as root system stiffness in N/m.

*Device Validation*

A validation stand was constructed consisting of a 1-inch (2.54 cm) square steel tube attached with a hinge to a base plate (**Supplemental Figure 3A**). A U-bolt was attached through the base plate at 44.9 mm distance from the hinge point and an eye bolt was threaded through the rod at height of 44.9 mm to generate a 45-deg angle for a 2.5 inch (6.35 cm) spring test. Two different springs were tested using the same approach detailed above. Specifically, the SMURF was attached and detached three times, and at each attachment tests were run three consecutive times. Based on the geometry of the testing stand, the displacement of the spring was calculated from the vertical displacement of the SMURF. Specifically, the hypotenuse of a right triangle was calculated at each displacement based on the initial SMURF height of 38.5 cm. The hypotenuse of the right triangle created by the spring attachment was then derived from the SMURF displacement calculated hypotenuse values. The slope of the force-displacement of the spring was then extracted as the measured spring rate (N/m).

*Repeatability of Root System Stiffness Measurements*

To determine if testing multiple times impacted the repeatability of the root system stiffness measurements *in planta*, two replicate plants of CML258 were subjected to repeat testing at 85 dap. The SMURF device was attached to the base of the plant approximately perpendicular to the row and three consecutive tests were run (A, B, and C) without removing the SMURF device (Stalk Position 1). The SMURF device was then removed from the plant, attached at a second position (Stalk Position 2), and tested three consecutive times (A, B, and C). This process was repeated for a total of three positions (Stalk Position 1, Stalk Position 2, and Stalk Position 3), with each position approximately 45-deg apart.

To determine if the time of day affected the root system stiffness measurements, three maize inbred lines (CML258, B73, and W22) were tested at 9:00 AM, 12:00 PM, and 4:00 PM Eastern Standard Time on the same day. In 2021, data was collected for B73 and W22 at 60 dap and at 85 dap for CML258. In 2022, data as collected at 101 dap for CML258.

*Root System Stiffness Measurements*

Root system stiffness data was measured for 11 hybrids with variable responses of root lodging in 2020, 2021, and 2022. A sample size analysis was performed with G*Power (Faul *et al.*, 2009) and determined that 5-6 replicates are required for statistical power of 0.05. Data was collected from at least two plot replicates and at least five plants per plot in 2021 and 2022. In 2020, data was collected from plants that did not lodge at 16 days after the storm (08/20/2020; 55 dap; R2 growth stage) and at the end of the season (09/25/2020; 91 dap; R5 growth stage). In 2021, data was collected from all hybrids at 83 dap (08/11/2021; R4 growth stage) and 117 dap (09/14/2021; R6 growth stage). In 2022, data was collected from all hybrids at 116 dap (09/16/2022; R6 growth stage). Cumulative growing degree day (GDD) data for our Newark Ag Farm location was downloaded from the NEWA growing degree day calculator (https://newa.cornell.edu/degree-day-calculator/) with Base 50ºF parameters (**Supplemental Figure 4**).

<u>Statistics</u>

All statistical analyses were performed using R ver. 4.0.2 (R Core Team ). To determine factors that influence root system stiffness, analysis of variance (ANOVA) models were used. Prior to running each ANOVA, a Shapiro-Wilk test was used to test the normality of residuals. If residuals were not drawn from a normal distribution, a Tukey's Ladder of Powers (*rcompanion* package ver. 2.4.1 (Mangiafico 2021)) transformation was used. To determine if variances were equal, a Levenes test (*car* package (Fox and Weisberg 2019)) was used. If a *p* value less than 0.05 was associated with our F statistic, a post-hoc Tukey honest significant difference (HSD) test was used to test all pairwise comparisons. All figures were generated with the ggplot2 (Wickham 2016) and the cowplot (Wilke 2020) package in R.

*Repeatability of Root System Stiffness Measurements*

To determine if repeat testing impacted the quantification of root system stiffness, a two-way ANOVA was used. Both test position (Stalk Position 1, Stalk Position 2, Stalk Position 3) and test order (A, B, C) were included as independent variables. To determine if time of testing impacted the quantification of root system stiffness, three models were tested. In the first model, a two-way ANOVA was run for 2021 data with only B73 and W22 genotypes, and genotype and time of day were included as independent variables. In the second model, a one-way ANOVA was run for CML258 in 2021 and time of day was included as an independent variable. In the third model, a one-way ANOVA was run for CML258 in 2022 and time of day was included as an independent variable.

*Variation of Root System Stiffness Measurements among Maize Hybrids*

To determine if hybrid impacted root system stiffness, one model was tested for each condition (five conditions total). Within each condition, a one-way ANOVA was used, where root system stiffness was the dependent variable and hybrid was the independent variable. The five conditions tested were: Condition 1 = Late planting (end of June), testing at the R2 growth stage, and 10 plant density; Condition 2 = Late planting (end of June), testing at the R5 growth stage, and 10 plant density; Condition 3 = Standard planting (end of May), testing at the R4 growth stage and 10 plant density; Condition 4 = Standard planting (end of May), testing at the R6 growth stage, and 10 plant density; Condition 5 = Standard planting (end of May), testing at the R6 growth stage, and 18 plant density.

*Variation of Root System Stiffness Measurements among Root Lodging Susceptibility Categories*

To determine if the root system stiffness metric could be used as a proxy for root lodging susceptibility, hybrids were grouped into three categories (low, moderate, or severe root lodging susceptibility) depending on the percentage of plants that experienced root lodging in 2020. To test this, one model was used for each condition (five conditions total; listed above). Within each condition, a one-way ANOVA was used where the root system stiffness was the dependent variable, and the root lodging category (low, moderate, or severe) was the independent variable.

*Variation in Aboveground Architectures and the Root System Stiffness in Response to Planting Density*

To determine if hybrid or density impacted plant phenotypes (stalk height, stalk diameter, root system stiffness, the ratio of root system stiffness/stalk height, the ratio of root system stiffness/stalk diameter), a two-way ANOVA was used with plant phenotype as the dependent variable and plant density and hybrid as the independent variables. Two-way ANOVAs were run individually for each dependent variable (stalk height, stalk diameter, root system stiffness, the ratio of root system stiffness/stalk height, the ratio of root system stiffness/stalk diameter).

## Results

<u>The SMURF quantifies the root system stiffness.</u>

The principle of the SMURF is based on the mechanics of a 4-bar linkage parallelogram, which forms a rigid parallelogram with two fixed links and two moving links (**Figure 2A-B**). The dual attachment points of the SMURF force the plant to act as a rigid bar. Thus, the attachment points between the plant and SMURF serve as fixed links, whereas the device foot on the ground and the root system serve as the moving links. To introduce motion into these links, the leadscrew extends to transmit a rotation at the opposite link (the root system) (**Figure 2C**). To maintain a rigid attachment, the foot of the SMURF slides outwards approximately 6 mm over the course of the testing cycle (**Supplemental Video 1**). The SMURF-measured stiffness is a systems-level characterization of root mechanics, which includes the resistance to uprooting and the resistance to rotation. We propose that most of this measurement comes from the resistance to rotation as opposed to uprooting-resistance. This expectation is based on the behavior of the system, where an uprooting force would cause the plant to move vertically upon SMURF displacement instead of rotating. This systems-level measurement describes the overall elastic behavior of the root system.

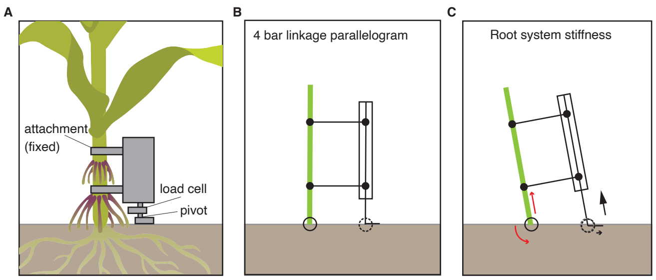


**Figure 2. Drawing of SMURF Mechanics.** A) The SMURF is attached to the base of the plant using two rigid arms. This attachment is fixed with no slippage vertically along the stalk. B) The mechanics of the SMURF is based on a 4-bar linkage parallelogram with fixed (closed circles) and moving links (open circles). C) The vertical extension of the leadscrew induces a rotation at the root system link. The measurement is based on both the resistance to uprooting and resistance to rotation (red arrows). To accommodate the mechanics, the foot of the SMURF slides horizontally (grey dotted line). Friction has been ignored to simplify the physics.

We have previously shown that the root system is best modeled as a rotational spring (Obayes *et al.*, 2022). Thus, to verify the ability of the SMURF to measure the mechanics of a spring (proxy for the root system) as opposed to the bar (proxy for the stalks), a validation stand was constructed (**Supplemental Figure 3A**). The results of the validation stand show that the SMURF can faithfully measure two spring rates (**Supplemental Figure 3B**). For a spring with a known spring rate of 4255 N/m, the SMURF measured spring rate is 4104 $\pm$ 326.6, and for a spring with a known spring rate of 6829 N/m, the SMURF measured spring rate is 6564 $\pm$ 32.8. In both cases, the average measured spring rate is within 4% of the known spring rate. These results show that the SMURF measured stiffness is directly related to spring rate.

<u>Root system stiffness measurements are reproducible across testing positions and time of day.</u>

The testing parameters and potential constraints of using the SMURF device were assessed by two independent analyses. First, the repeatability of measurements taken at the same position (called Test Order: A, B, C) and at different attachment positions (called Test Position: Stalk Position 1, Stalk Position 2, Stalk Position 3) were assessed. When plants were tested at the same stalk position (without removing the device), each consecutive test increased the root system stiffness ($p < 0.05$, **Figure 3A, Supplemental Tables 1-2**). The second test (Test Order B) had an average increase of 26% compared to the first test (Test Order A) and the third test (Test Order C) had an average increase of 5% compared to the second test (Test Order B). These small increases in root system stiffness may be due to the slight loosening of the root-soil contacts in that measurement plane or due to mechanical alignment of the device materials (e.g., fiber alignment of the straps). When the SMURF device was removed and reattached at a new position, the root system stiffness did not differ between Test Position within the same Test Order ($p > 0.05$, **Figure 3A**). This data shows that the position of device attachment on the plant stalk does not influence the outcome.

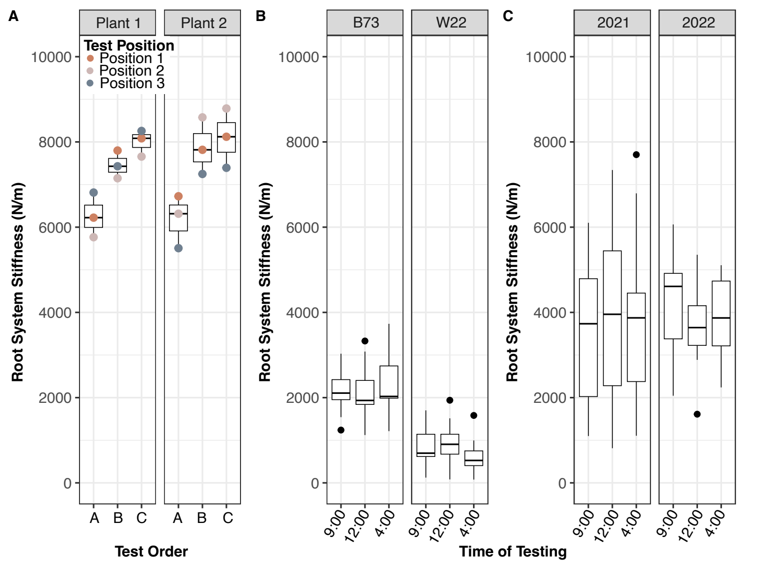


**Figure 3. Parameterization of SMURF Root System Stiffness Measurements.** (A) A SMURF device was attached to three test positions on the base of two separate plants (Stalk Position 1, Stalk Position 2 or Stalk Position 3) and the root system stiffness was measured three consecutive times at each position (Test Order A, B, or C; A=First Test, C=Last Test). A two-way ANOVA (Test Position*Test Order) showed that regardless of plant replicate, the root system stiffness was impacted by test order ($p < 0.05$) but not test position. (B) Root system stiffness was quantified at 9:00A, 12:00P, and 4:00P EST in 2021 for B73 and W22. A two-way ANOVA (Genotype*Time of Day) showed that there was no statistical difference in root system stiffness due to time of day, however genotypes (B73 and W22) did differ for root system stiffness ($p < 0.05$). (C) Root system stiffness was quantified at 9:00A, 12:00P, and 4:00P EST in 2021 and 2022 for CML258. A one-way ANOVA (Time of Day) within years showed that there was no statistical difference in root system stiffness in either year. (B-C) Black dots illustrate outliers.

We have previously shown that stalk mechanics are variable across the time of day, with higher stiffness values in the morning compared to the afternoon (Reneau *et al.*, 2020). To determine if the time of day similarly impacts the root system stiffness, measurements were taken at 9:00 AM, 12:00 PM, and 4:00 PM for three inbred lines (CML258, B73, and W22). In contrast to stalk mechanics, the time of day did not impact root system stiffness values regardless of genotype ($p > 0.05$, **Figure 3B and 3C**, **Supplemental Tables 3-4**). However, there were differences in root system stiffness between genotypes ($p < 0.05$, **Figure 3B**, **Supplemental Table 4**), demonstrating the potential utility of this metric to evaluate root system mechanics.

Root system stiffness varies among maize hybrids.

A collection of 11 hybrids that were previously shown to vary in root lodging susceptibility (Tirado *et al.*, 2021) were assessed across multiple years, growth stages, and planting densities. In 2020, Tropical Storm Isaias caused root lodging (**Supplemental Figure 1A-B**) and the different root lodging susceptibility for these hybrids was confirmed at our field site. Root lodging susceptibility varied from 0% (no root lodging within plot) to 100% root lodging (all plants root lodged within plot), with a population average of 48.7% ± 38.2%.

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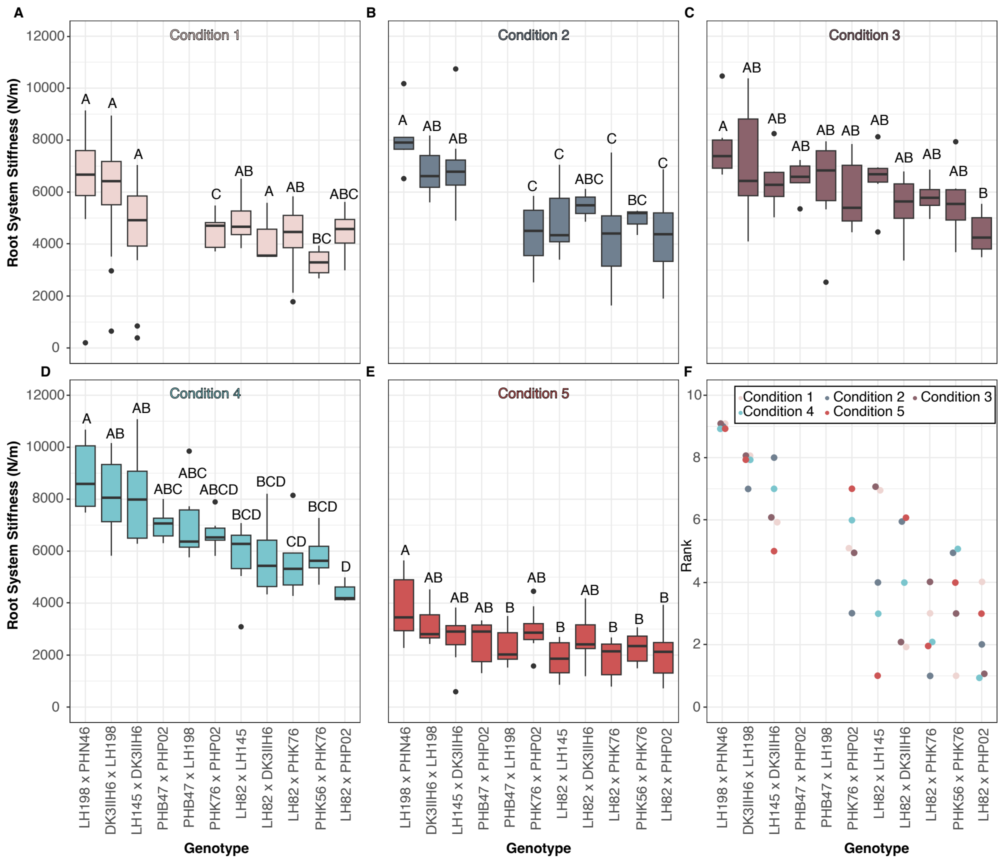


**Figure 4. Maize hybrids have variable root system stiffness.** (A-E) The root system stiffness was measured with SMURF devices for 11 hybrids across three years (2020, 2021 and 2022), multiple growth stages (R2, R4, R5, R6) and multiple plant densities (10 plant density and 18 plant density). Statistics were run within a condition and regardless of condition the root system stiffness varied by hybrids ($p < 0.05$). Letters represent Tukey HSD groups and hybrids that share a letter within a condition are not statistically different from one another. Black dots indicate outliers. Boxes are colored according to Panel F. (A) Condition 1: Late plant, R2 growth stage, 10 plant density. (B) Condition 2: Late plant, R5 growth stage, 10 plant density. (C) Condition 3: Standard plant, R4 growth stage, 10 plant density. (D) Condition 4: Standard plant, R6 growth stage, 10 plant density. (E) Condition 5: Standard plant, R6 growth stage, 18 plant density. (F) Within each condition, hybrids were ordered from highest root system stiffness to lowest root system stiffness and assigned a rank from 1-9 with 9 being the highest root system stiffness compared to the other hybrids within that condition and 1 being the lowest root system stiffness compared to other hybrids within that condition. Hybrids PHB47 x PHP02 and PHB47 x LH198 were not included in the ranking due to 100% lodging in 2020 which resulted in missing data for two conditions (Condition 1 and Condition 2).

The SMURF device was used to evaluate the differences in root system stiffness among these hybrids. Specifically, plants were assessed across three years and three growth stages (referred to as conditions). Regardless of condition, there was a significant effect of hybrid on root system stiffness measurements ($p < 0.05$, **Figure 4A-E**, **Supplemental Tables 5-6**; $p < 0.05$). Further analysis shows that when ranking the hybrids from highest root system stiffness (rank 9) to lowest root system stiffness (rank 1) within a condition, hybrids rank similarly across multiple conditions (**Figure 4F**). This demonstrates that the SMURF can be used to evaluate differences in root system stiffness among hybrids under multiple conditions.\

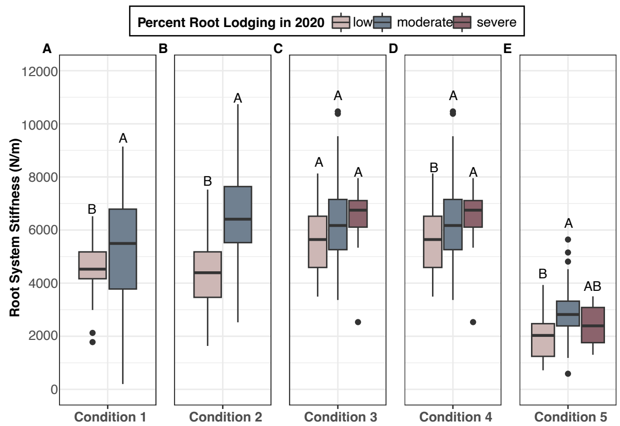


**Figure 5. Increased root lodging is associated with a higher root system stiffness.** (A-E) The root system stiffness was quantified with SMURF devices for 11 hybrids across three years (2020, 2021, and 2022), multiple growth stages (R2, R4, R5, and R6) and multiple plant densities (10 plant density and 18 plant density). Hybrids were grouped into lodging categories according to their response to Tropical Storm Isaias in August of 2020. Hybrids were classified as low when the root lodging response from both plots was more than 1 standard deviation below the population mean for root lodging and were considered severe when the root lodging response from both plots was more than 1 standard deviation above the population mean for root lodging. A one-way ANOVA was run within each condition to evaluate if there was a statistical significance in root system stiffness explained by the root lodging categories. Within each condition, categories that share a letter are not statistically different from one another ($p<0.05$). Black dots indicate outliers.
Condition 1: Late plant, R2 growth stage, 10 plant density. Condition 2: Late plant, R5 growth stage, 10 plant density. Condition 3: Standard plant, R4 growth stage, 10 plant density. Condition 4: Standard plant, R6 growth stage, 10 plant density. Condition 5: Standard plant, R6 growth stage, 18 plant density.

<u>A higher root system stiffness is associated with increased root lodging</u>.

To determine if the root system stiffness can be used as a proxy for root lodging susceptibility, hybrids were grouped into three categories (low, moderate, severe) depending on the percentage of plants across both plots that experienced root lodging in 2020. Root system stiffness measurements were obtained at two growth stages after the lodging event, and data collection was limited to plants that did not lodge. At both growth stages (Condition 1 and Condition 2), the moderate root lodging hybrids had a higher root system stiffness than the low root lodging hybrids ($p < 0.05$, **Figure 5A-B**, **Supplemental Tables 7-8**). Since SMURF measurements were only taken after the root lodging event, it was not clear if these results were impacted by the plants recovering from the storm or were inherent to the hybrid susceptibility to root lodging. Therefore, the root lodging categories from 2020 were used to assess the differences in root system stiffness in subsequent conditions. Again, hybrids with moderate or severe root lodging in 2020 had a higher root system stiffness compared to the low root lodging hybrids regardless of condition ($p < 0.05$, **Figure 5C-E, Supplemental Table 8**). Collectively, these results show that root system stiffness is related to the plant susceptibility to root lodging and that a higher root system stiffness is associated with increased root lodging.

<u>As planting density increases, the root system stiffness decreases.</u>

The relative relationship of root system stiffness measurements among hybrids was consistent across conditions including two planting densities (**Figure 4 and Figure 5**). Prior studies have shown a reduction in maize root system growth in response to increasing planting densities (Liu *et al.*, 2012), which should reduce the root system stiffness. To test this hypothesis, two hybrids (LH82 x DK3IIH and LH82 x PHP02) were planted at four densities (6, 12, 18, and 24 plants/plot). Increasing planting density increased stalk height ($p < 0.05$, **Figure 6A**) and decreased stalk diameter ($p < 0.05$, **Figure 6B**, **Supplemental Tables 9-10**) consistent with the expected induction of a shade avoidance response. Additionally, root system stiffness decreased as planting density increased ($p < 0.05$, **Figure 6C**, **Supplemental Tables 9-10**). These data show that root system stiffness is related to the size of the root system, which can be modulated by planting density.

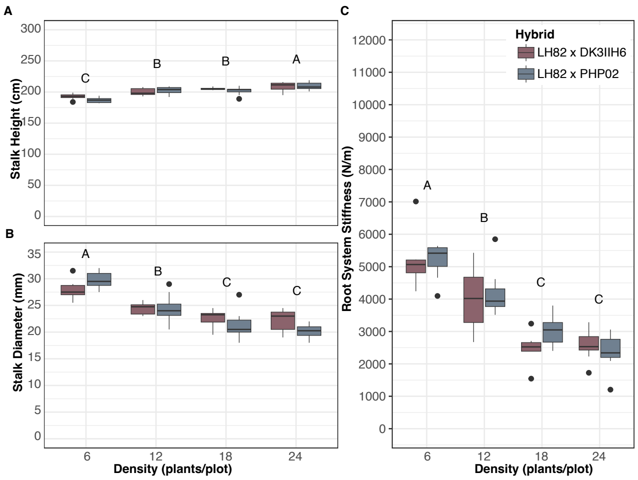


**Figure 6. As planting density increases, plant architecture changes.** Two hybrids (LH82 x DK3IIH and LH82 x PHP02) were planted at four densities (6, 12, 18, and 24 plants/plot) and assessed for stalk height, stalk diameter, and root system stiffness. (A) As planting density increases, stalk height increases. A two-way ANOVA (Hybrid*Density) revealed that the hybrid did not impact stalk height, but planting density did impact stalk height (p < 0.05). (B) As planting density increases, stalk diameter decreases. A two-way ANOVA (Hybrid*Density) revealed that the hybrid did not impact stalk diameter, but planting density did impact stalk diameter (p < 0.05). (C) As planting density increases, the root system stiffness decreases. A two-way ANOVA (Hybrid*Density) revealed that the hybrid did not impact root system stiffness, but planting density did impact the root system stiffness (p < 0.05). (A-C) Within each panel (stalk height, stalk diameter, or root system stiffness), densities that share a letter do not significantly differ (Tukey HSD assessed at p < 0.05). Black dots indicate outliers.

## Discussion

While tools that measure stalk stiffness, stalk strength, and root strength have been reported (Erndwein *et al.*, 2020), this is the first tool that measures root system stiffness in large grain crops. When used in combination with other tools, the SMURF enables a comprehensive understanding of the integrated plant mechanics and lodging (**Figure 1**). The SMURF is a non-destructive, lightweight (1.4 kg), portable (39 cm (l) x 21 cm (w) x 5 cm (d)), and high throughput (< 2 min/test) tool that will facilitate proactive screening for root lodging resistant genotypes.

While the SMURF fills a gap in our ability to quantify plant mechanics, the SMURF, and all current biomechanical devices, rely on static mechanics (Erndwein *et al.*, 2020). In other words, there is the artificial application of a displacement and the measurement of the forces resisting that displacement (Niklas and Spatz 2012). However, the wind component is dynamic loading, and this dynamic loading likely leads to a cumulative thigmotropic response of the plants. To date, there is a lack of tools to assess plant dynamics in a field setting.

Accepted Manuscript
Version of Record at: https://doi.org/10.1093/jxb/erae465

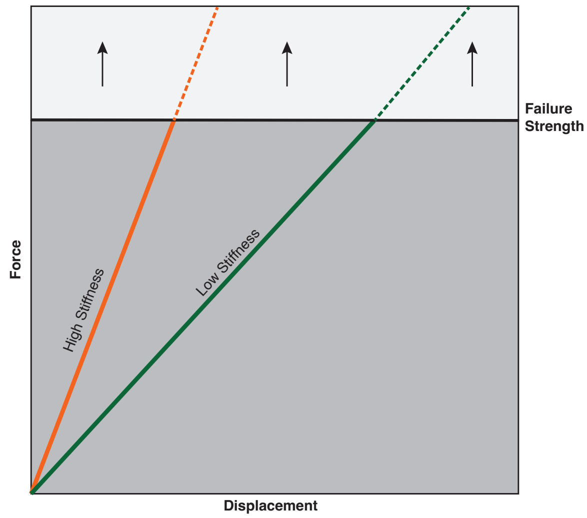


**Figure 7. Higher root system stiffness causes failure strength to be reached at lower displacements.** Root mechanics is encompassed by both the stiffness and strength of the root system. Regardless of the overall failure strength, a root system with a higher stiffness (orange line) will reach the failure strength at a lower displacement than a root system with a lower stiffness (green line).

Findings from this study show that the hybrids with a higher root system stiffness are associated with increased root lodging susceptibility. While it may be intuitive to think that a stiffer base would serve as a better anchor, instead a stiffer base means that a plant will reach the failure strength at lower displacements (**Figure 7**). Thus, regardless of the failure strength of the root system, a stiffer root system will fail more frequently than a less stiff root system assuming the stalk transmits equal displacement.

Root lodging is the outcome of the externally applied bending moment (created by a combination of wind speed, plant height, and the drag characteristics of the plant (Stubbs *et al.*, 2023)) exceeding the root system strength. A lower root system stiffness can withstand greater displacements before failure, but if the failure strength is lowered or the bending moment is increased, root lodging can still occur. For example, we show that the root system stiffness decreased with increasing planting densities, but we know that increasing planting density is associated with increased root lodging (Zhang *et al.*, 2022). In this case, while we are optimizing the root system stiffness, we are lowering the root strength and increasing the externally applied bending moment by changing plant height. Indeed, analysis of the ratio between the root system stiffness and aboveground architectures shows that plants grown at higher densities must support approximately 2 times the stalk height and 1.5 times the diameter as their counterparts at lower densities (**Figure 5**, **Supplemental Tables 11-12**).

Root system mechanics are determined by plant and soil characteristics, including root architecture, individual root mechanics, soil mechanics, and soil moisture. A recent study showed that root architecture explains 45% of the variation in root lodging, and suggests plant composition (e.g., lignin) is equally important (Zheng *et al.*, 2023). A non-destructive tool, such as SMURF, enables quantification of the specific plant and soil contributions to root anchorage, thus paving the way towards a comprehensive understanding of the biology of root lodging.

## Supplementary Data

**Supplemental Figure S1.** Root lodging susceptibility is variable among maize hybrids and random in the field.

**Supplemental Figure S2.** The SMURF device consists of a physical body, electronics, powertrain, sensors, and software.

**Supplemental Figure S3.** A device validation stand was constructed and shows that the SMURF device can distinguish between the stiffness of different springs.

**Supplemental Figure S4.** The cumulative growing degree day (GDD) was assessed for the three years of data collected.

**Supplemental Figure S5.** Root system stiffness varies with stalk height and stalk diameter.

**Supplemental Table 1.** A two-way analysis of variance (ANOVA) showed that stalk position did not impact root system stiffness ($p > 0.05$), while test order did impact root system stiffness ($p < 0.05$).

**Supplemental Table 2.** When plants were tested at the same stalk position (without removing the device), each consecutive test increased the root system stiffness ($p<0.05$) while position on the stalk did not ($p > 0.05$).

**Supplemental Table 3.** Regardless of genotype or condition, analyses of variances show that time of testing does not impact root system stiffness ($p > 0.05$)

**Supplemental Table 4.** Data from 2021 show that on average B73 has a higher root system stiffness than W22 ($p<0.05$).

**Supplemental Table 5.** Regardless of condition, analyses of variances show that maize hybrid impacts the root system stiffness ($p>0.05$)

**Supplemental Table 6.** Within each condition, genotypic differences in root system stiffness remain similar.

**Supplemental Table 7.** Regardless of condition, analysis of variances show that root lodging category impacts the root system stiffness ($p>0.05$)

**Supplemental Table 8.** Within each condition, hybrids that were categorized as having low levels of root lodging had lower root system stiffness values compared to those that were categorized has having moderate or high levels of root lodging.

**Supplemental Table 9.** Two-way analysis of variance tests showed that plant density impacted the root system stiffness ($p<0.05$), plant height ($p<0.05$), and stalk diameter ($p<0.05$), but genotype did not ($p>0.05$ for all three)

**Supplemental Table 10.** As plant density increased stalk height increased, but stalk diameter and root system stiffness decreased.

**Supplemental Table 11.** Two-way analysis of variance tests showed that plant density impacted the ratio of the root system stiffness to stalk height ($p<0.05$), and the ratio of the root system stiffness to stalk diameter ($p<0.05$).

**Supplemental Table 12.** As plant density increased, both the ratio of the root system stiffness to stalk height and the ratio of root system stiffness to stalk diameter decreased.

**Supplemental Video 1.** The SMURF is attached to the plant at the two arms as low as possible on the stalk while avoiding brace roots.

## Declarations

Consent for publication

Not applicable.

Competing Interests

Data in this paper was generated using the SMURF device. J.W.R. and E.E.S. are inventors on a patent application claiming the SMURF device filed by the University of Delaware.

Funding

This work was supported by a United States Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA) Postdoctoral Fellowship awarded to ANH (grant no. 2022-67012-36840).

Acknowledgements

We thank members of the Sparks Lab (Irene I Ikiriko, K. Thanduanlung, Eva Birtell, Het Patel, Dave Griffin, Kishan Biradar, and Emilia Pierce) for providing feedback during manuscript preparation. We thank Dr. Adam Stager for assembling the SMURF 1.0, which formed the basis for the device detailed in this manuscript and Dr. David Burris for helpful discussion on the interpretation of the mechanics. We thank the Computer Science capstone team, especially Will Koenig, for developing a prototype of the SMURF iPad application software. We thank Dr. Ron Ferris for funding high-school interns and co-authors (Taran Kermani and Therese Kim). We thank Dr. Lindsay Erndwein of Illustrations by LindZeaMays for illustrating the plant in Figure 1 and Supplemental Figure 2. We thank Dr. Candice Hirsch (University of Minnesota) for providing the hybrid seed for the initial studies. We thank two anonymous reviewers for providing manuscript feedback.

Author Contributions

J.W.R. and E.E.S. conceptualized the project and developed the SMURF. J.W.R., T.W., T.A.K., and T.T.K. collected the data. A.N.H. and E.E.S. analyzed and interpreted the data. J.W.R. and J.C. developed the SMURF software and Bluetooth integration. All authors contributed to the writing and/or editing of the manuscript.

Availability of Data and Materials

All raw data, processed data, and R scripts to analyze data are available on GitHub: https://github.com/EESparksLab/Hostetler_Reneau_et_al_2023

A detailed protocol on how to use the SMURF device is available at protocols.io: dx.doi.org/10.17504/protocols.io.yxmvmer9ng3p/v1

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## Figure Legends

**Figure 1. Schematic of the plant mechanical basis of lodging.** When wind interacts with a plant, it is perceived by the stalk and induces stalk displacement. If the wind-induced displacement exceeds the stalk strength, the stalk will fail, called stalk lodging. For wind-induced displacements that are less than the stalk strength, the displacement translates into root displacement. If the wind-stalk-induced root displacement exceeds the root strength, the root will fail, called root lodging. For both stalk and root, strength has been reported as the force to complete failure (e.g. breaking or uproot), and stiffness represents the rate at which the displacement reaches that force. We argue that any plastic deformation indicates structural failure and will contribute to lodging. There are currently tools available to measure stalk stiffness, stalk strength, and root strength, but not root stiffness.

**Figure 2. Drawing of SMURF Mechanics.** A) The SMURF is attached to the base of the plant using two rigid arms. This attachment is fixed with no slippage vertically along the stalk. B) The mechanics of the SMURF is based on a 4-bar linkage parallelogram with fixed (closed circles) and moving links (open circles). C) The vertical extension of the leadscrew induces a rotation at the root system link. The measurement is based on both the resistance to uprooting and resistance to rotation (red arrows). To accommodate the mechanics, the foot of the SMURF slides horizontally (grey dotted line). Friction has been ignored to simplify the physics.

**Figure 3. Parameterization of SMURF Root System Stiffness Measurements.** (A) A SMURF device was attached to three test positions on the base of two separate plants (Stalk Position 1, Stalk Position 2 or Stalk Position 3) and the root system stiffness was measured three consecutive times at each position (Test Order A, B, or C; A=First Test, C=Last Test). A two-way ANOVA (Test Position*Test Order) showed that regardless of plant replicate, the root system stiffness was impacted by test order ($p < 0.05$) but not test position. (B) Root system stiffness was quantified at 9:00A, 12:00P, and 4:00P EST in 2021 for B73 and W22. A two-way ANOVA (Genotype*Time of Day) showed that there was no statistical difference in root system stiffness due to time of day, however genotypes (B73 and W22) did differ for root system stiffness ($p <0.05$). (C) Root system stiffness was quantified at 9:00A, 12:00P, and 4:00P EST in 2021 and 2022 for CML258. A one-way ANOVA (Time of Day) within years showed that there was no statistical difference in root system stiffness in either year. (B-C) Black dots illustrate outliers.

**Figure 4. Maize hybrids have variable root system stiffness.** (A-E) The root system stiffness was measured with SMURF devices for 11 hybrids across three years (2020, 2021 and 2022), multiple growth stages (R2, R4, R5, R6) and multiple plant densities (10 plant density and 18 plant density). Statistics were run within a condition and regardless of condition the root system stiffness varied by hybrids ($p < 0.05$). Letters represent Tukey HSD groups and hybrids that share a letter within a condition are not statistically different from one another. Black dots indicate outliers. Boxes are colored according to Panel F. (A) Condition 1: Late plant, R2 growth stage, 10 plant density. (B) Condition 2: Late plant, R5 growth stage, 10 plant density. (C) Condition 3: Standard plant, R4 growth stage, 10 plant density. (D) Condition 4: Standard plant, R6 growth stage, 10 plant density. (E) Condition 5: Standard plant, R6 growth stage, 18 plant density. (F) Within each condition, hybrids were ordered from highest root system stiffness to lowest root system stiffness and assigned a rank from 1-9 with 9 being the highest root system

stiffness compared to the other hybrids within that condition and 1 being the lowest root system stiffness compared to other hybrids within that condition. Hybrids PHB47 x PHP02 and PHB47 x LH198 were not included in the ranking due to 100% lodging in 2020 which resulted in missing data for two conditions (Condition 1 and Condition 2).

**Figure 5. Increased root lodging is associated with a higher root system stiffness.** (A-E) The root system stiffness was quantified with SMURF devices for 11 hybrids across three years (2020, 2021, and 2022), multiple growth stages (R2, R4, R5, and R6) and multiple plant densities (10 plant density and 18 plant density). Hybrids were grouped into lodging categories according to their response to Tropical Storm Isaias in August of 2020. Hybrids were classified as low when the root lodging response from both plots was more than 1 standard deviation below the population mean for root lodging and were considered severe when the root lodging response from both plots was more than 1 standard deviation above the population mean for root lodging. A one-way ANOVA was run within each condition to evaluate if there was a statistical significance in root system stiffness explained by the root lodging categories. Within each condition, categories that share a letter are not statistically different from one another ($p<0.05$). Black dots indicate outliers. Condition 1: Late plant, R2 growth stage, 10 plant density. Condition 2: Late plant, R5 growth stage, 10 plant density. Condition 3: Standard plant, R4 growth stage, 10 plant density. Condition 4: Standard plant, R6 growth stage, 10 plant density. Condition 5: Standard plant, R6 growth stage, 18 plant density.

**Figure 6. As planting density increases, plant architecture changes.** Two hybrids (LH82 x DK3IIH and LH82 x PHP02) were planted at four densities (6, 12, 18, and 24 plants/plot) and assessed for stalk height, stalk diameter, and root system stiffness. (A) As planting density increases, stalk height increases. A two-way ANOVA (Hybrid*Density) revealed that the hybrid did not impact stalk height, but planting density did impact stalk height ($p < 0.05$). (B) As planting density increases, stalk diameter decreases. A two-way ANOVA (Hybrid*Density) revealed that the hybrid did not impact stalk diameter, but planting density did impact stalk diameter ($p < 0.05$). (C) As planting density increases, the root system stiffness decreases. A two-way ANOVA (Hybrid*Density) revealed that the hybrid did not impact root system stiffness, but planting density did impact the root system stiffness ($p < 0.05$). (A-C) Within each panel (stalk height, stalk diameter, or root system stiffness), densities that share a letter do not significantly differ (Tukey HSD assessed at $p < 0.05$). Black dots indicate outliers.

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