

Biotechnology for Fuels and Chemicals

The Twenty-Sixth Symposium

Presented as Volumes 121–124
of Applied Biochemistry and Biotechnology

Proceedings of the Twenty-Sixth Symposium
on Biotechnology for Fuels and Chemicals
Held May 9 – May 12, 2004, in Chattanooga, TN

Sponsored by

U.S. Department of Energy's Office of the Biomass Program
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Applied Biochemistry and Biotechnology

Volumes 121–124, Complete, Spring 2005

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Applied Biochemistry and Biotechnology is abstracted or indexed regularly in *Chemical Abstracts*, *Biological Abstracts*, *Current Contents*, *Science Citation Index*, *Excerpta Medica*, *Index Medicus*, and appropriate related compendia.

Introduction to the Proceedings of the Twenty-Sixth Symposium on Biotechnology for Fuels and Chemicals

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The Twenty-Sixth Symposium on Biotechnology for Fuels and Chemicals was held May 9 – May 12, 2004 in Chattanooga, Tennessee. The symposium continues to have an interdisciplinary focus on bioprocessing, and remains the preeminent forum for exchange of information and technology, updating of current trends in biotechnology and bringing together active participants and organizations in the area of sustainable fuels and chemicals production. This annual symposium focuses on advances in biotechnology to produce high-volume, low-price products from renewable resources, as well as to improve the environment. Session topics included advanced feedstock production and processing, enzyme and microbial biocatalysts, bioprocess research and development, opportunities in biorefineries, commercialization of bio-based products, and a number of other special topics sessions.

While advances in commercialization of bioproducts continued apace this year and excitement remains high, there was a ‘momentary catching of our collective breath’ with examining the hard tasks of implementing commercialization and considering what might be the next breakthroughs and the next bioproducts. In particular, there remains a need to move beyond corn sugar as the primary feedstock into lignocellulosics.

Participants from academic, industrial, and government venues convened to discuss the latest research breakthroughs and results in biotechnology to improve the economics of producing fuels and chemicals. The total of 343 attendees represented a 15% increase over the 2002 conference in Gatlinburg. Of this total, about 20% were students (and another 30% from academia), 30% from industry, and 20% from government. The

increased attendance required concurrent sessions for the 50 oral presentations and 236 submitted posters. [details at www.ct.ornl.gov/symposium].

Almost 30% of the attendees were international, showing the strong worldwide interest in this area. Nations represented included Australia, Austria, Belgium, Brazil, Canada, China, Denmark, England, Finland, France, Hungary, Japan, Mexico, The Netherlands, New Zealand, Portugal, Russia, South Africa, South Korea, Spain, Sweden, Turkey and Venezuela, as well as the United States.

Bioconversion of renewable resources into fuels has focused on the conversion of lignocellulose into sugars and the conversion of sugars into products and fuels. This focus has expanded into examining the concept of the biorefinery – the integrated production of multiple products and energy at one site. This was a theme for many talks and presentations. However, several new areas gained resurgent interest in presentations – the gasification of biomass and its subsequent conversion into fuels or chemicals as well as two areas that were emphasized in Special Topics discussions.

The conversion of the third constituent of biomass, lignin, was featured in a Special Topic Session entitled “Lignin from Biorefineries: Chemical and Biochemical Perspectives and Applications” chaired by Abhijeet Borole of ORNL and Kendall Pye of Lignol Innovations Corporation.

A very different bioconversion challenge was presented in the second Special Topic, “Biohydrogen: The Challenges and the Possible Future,” chaired by James W. Lee of ORNL. This session presented updates on improving the rate, concentration, and efficiency of biological hydrogen production in light and dark reactions as well as perspectives on the process design for these systems.

The 2004 Charles D. Scott award for Distinguished Contributions in the field of Biotechnology for Fuels and Chemicals was presented to Guido Zacchi. Dr. Zacchi has worked on implementation of bioenergy and biomass conversion for over two decades, primarily at Lund University in Sweden where he received his Ph.D. and where he continues to lead an active program today. He has over a hundred publications and has been an active participant and leader of the symposia. He participated in the first Swedish biomass-to-ethanol project in the 1980s and now operates a national process development unit which has resulted in a substantial increase in ethanol yield from softwood. This unit’s results are being used in a full pilot to begin in May 2004. This award was created to honor Dr. Charles D. Scott, founder of this Symposium and its chair for the first ten years.

Session Chairs

Session 1A: *Feedstock Supply, Logistics, Processing and Composition*

Chairs: Hans-Joachim G. Jung, USDA/ARS,
St. Paul, Minnesota
David Thompson, INEEL, Idaho Falls, ID

Session 1B: *Enzyme Catalysis and Engineering*

Chairs: Timothy Dodge, Genencor International,
Palo Alto, California
Gisella M. Zanin, State University of Maringa,
Maringa, Brazil

Session 2: *Microbial Catalysis and Metabolic Engineering*

Chairs: Johannes van Dijken, Delft University
of Technology, The Netherlands
Greg Luli, B.C. International, Alachua, FL

Session 3: *Bioprocessing – Including Separations*

Chairs: Susan M. Hennessey, DuPont, Wilmington, DE
Peter van Walsum, Baylor University, Waco, TX

Session 4: *More than Technology – Finance and Policy to Create the Biorefinery*

Chair: James Stoppert, Cargill, Inc., Wayzata, MN
Todd Werpy, Pacific Northwest National Laboratory,
Richland, WA

Session 5: *Biobased Industrial Chemicals*

Chairs: Charles Abbas, Archer-Daniels Midland, Decatur, IL
Paul Roessler, The Dow Chemical Company,
San Diego, CA

Session 6A: *Biomass Pretreatment and Hydrolysis*

Chairs: Bruce Dien, USDA/NCAUR, Peoria, IL
Quang Nguyen, Abengoa Bioenergy Corporation,
Chesterfield, MO

Session 6B: *Plant Biotechnology and Feedstock Genomics*

Chairs: Daniel Jones, USDA/CSREES, Washington, DC
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Acknowledgments

The continued success of the Symposium is due to the many participants, organizers, and sponsors, but is also a success and pleasure due to the diligent and creative staff. In particular, Nancy Watlington of ORNL, the conference secretary and Liz Willson and Jim Duffield of NREL, assistant conference secretaries, provided advice, persistence and unfailing good humor. Dr. John Barton contributed greatly to the website design and implementation. Other staff assisting ORNL included Norma Cardwell, Angie Fincher, Norm Kurtz, Ann Luffman, Tony McBee, Whitney Ridenour and Julie Subsavad.

Oak Ridge National Laboratory is operated for the US Department of Energy by UT-Battelle, LLC under contract DE-AC05-00OR22725.

The National Renewable Energy Laboratory is operated for the US Department of Energy by Midwest Research Institute, Battelle, and Bechtel under contract DE-AC36-99GO10337.

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5. "Proceedings of the Fifth Symposium on Biotechnology for Fuels and Chemicals" (1983), *Biotechnol. Bioeng. Symp.* **13**.
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20. "Proceedings of the Twentieth Symposium on Biotechnology for Fuels and Chemicals" (1999), *Appl. Biochem. Biotechnol.* 77–79.
21. "Proceedings of the Twenty-first Symposium on Biotechnology for Fuels and Chemicals" (2000), *Appl. Biochem. Biotechnol.* 84–86.
22. "Proceedings of the Twenty-second Symposium on Biotechnology for Fuels and Chemicals" (2001), *Appl. Biochem. Biotechnol.* 91–93.
23. "Proceedings of the Twenty-third Symposium on Biotechnology for Fuels and Chemicals" (2002), *Appl. Biochem. Biotechnol.* 98–100.
24. "Proceedings of the Twenty-Fourth Symposium on Biotechnology for Fuels and Chemicals" (2003), *Appl. Biochem. Biotechnol.* 105–108.
25. "Proceedings of the Twenty-fifth Symposium on Biotechnology for Fuels and Chemicals" (2004), *Appl. Biochem. Biotechnol.* 113–116.

This symposium has been held annually since 1978. We are pleased to have the proceedings of the Twenty-sixth Symposium currently published in this special issue to continue the tradition of providing a record of the contributions made.

The Twenty-seventh Symposium will be May 1-4, 2005 in Denver, Colorado. For more information on the 26th and 27th Symposia, visit the following websites: [<http://www.ct.ornl.gov/symposium>] and [http://nrel.gov/biotech_symposium]. We encourage comments or discussions relevant to the format or content of the meetings.

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SESSION 1A

FEEDSTOCK SUPPLY, LOGISTICS, PROCESSING, AND COMPOSITION

Session 1A

Feedstock Supply, Logistics, Processing, and Composition

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A cost effective and sustainable supply of biomass feedstocks is a critical component of a viable biorefinery industry that is capable of making a credible impact on petroleum displacement. Feedstock costs can amount to a very significant fraction of the cost of the final biorefinery product. Thus, the reduction of the costs of feedstock production, harvest, collection, transportation, storage, and preprocessing can have a direct and positive effect on the overall viability of a given biorefinery. In addition, the feedstock and technology choices that are made for maintaining a sustainable biomass supply will have important implications not only for the biorefinery industry, but also for society as a whole. This session focused on feedstock supply, logistics, processing and composition, all of which are important elements of the feedstock supply chain.

Ken Vogel of USDA-ARS began the program with a discussion of two major potential biomass energy crops, alfalfa and switchgrass. He highlighted the environmental benefits of including these crops in the agricultural landscape, as well as the unique agronomic traits that make these species attractive for biomass production. Because the chemical composition of biomass is critical in determining its potential for conversion to ethanol, the next presentation by Bonnie Hames of NREL on this topic was fitting. She described the new and modified set of analytical procedures developed by NREL to more accurately and completely account for the many chemical components of herbaceous biomass. The biomechanical properties of straw and corn stover were described by Christopher Wright of INL. Designers of processing facilities for handling biomass should take note of the significantly different compression modulus which were found within and among straws from wheat and barley varieties. Michael Montross of the University of Kentucky reported on the results of making adjustments to a conventional grain combine for use in one-pass corn grain and stover harvest. These results offered the possibility of one-pass harvesting, partial fractionation of the stover, without expensive equipment

redesign. Ensiling wheat straw was investigated by Joni Barnes of INL as an alternative method for storage and preservation of biomass. She reported that silage inoculant, moisture, and free sugar additions were necessary to stabilize polysaccharide composition in wheat straw during storage via ensiling. This effect was primarily due to the requirement for rapid and maintained reduction of pH. Use of slurries to transport and partially saccharify corn stover was evaluated through mathematical models presented by Amit Kumar of the University of Alberta. The session concluded with a cautionary presentation by Wallace Wilhelm of USDA-ARS that warned against the loss of soil organic matter from too much residue removal when harvesting biomass crops. Prolonged residue removal was reported to reduce soil organic matter and subsequent yields of crops. Papers presented as part of the poster session contributed additional insights on biomass production, compositional analysis, and biomechanical properties.

Biomechanics of Wheat/Barley Straw and Corn Stover

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Abstract

The lack of understanding the mechanical characteristics of cellulosic feedstocks is a limiting factor in economically collecting and processing crop residues, primarily wheat and barley stems and corn stover. Several testing methods—compression, tension, and bend—were investigated to increase the understanding of the biomechanical behavior of cellulosic feedstocks. Biomechanical data from these tests can provide required input to numerical models and help advance harvesting, handling, and processing techniques. In addition, integrating the models with the complete data set from this study can identify potential tools for manipulating the biomechanical properties of plant varieties in such a manner as to optimize their physical characteristics to produce higher-value biomass and more energy-efficient harvesting practices.

Index Entries: Modulus of elasticity; biomechanics; wheat straw; corn stover; feedstock development.

Introduction

The vision for a viable bioenergy and bioproducts industry in the United States by 2030 estimates that 1 billion dry tons of sustainable lignocellulosic feedstock will be needed annually (1). Meeting this goal will require a wide variety of feedstock streams as inputs to biorefineries and power plants. Improved harvesting, processing, and bulk handling systems that are capable of separating the more valuable components and densifying the material for transportation and processing will need to be developed. Successfully designing and developing these systems requires a fundamental knowledge of the biomechanical properties and characteristics of feedstock.

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The importance of biomechanical data has long been recognized (2,3). However, the ability to characterize the physical properties of biomass in a manner that allows estimation of the energy consumption and power requirements of engineered feedstock systems has not been effectively addressed. In fact, the current biomechanical property data are generally limited to one or two varieties and do not attempt to apply the results to broad-scale harvesting, processing, and bulk handling systems. In addition, intervariety comparisons have not been widely investigated to determine the potential sources of mechanical variations. These relationships are necessary to develop predictive models that can potentially improve the effectiveness and efficiencies of these systems. Furthermore, intervariety comparisons can help connect the mechanical behavior of specific plant components to particular loading configurations, providing a path forward for genetically manipulating the varieties in order to optimize their macroscopic and microscopic characteristics.

Addressing the goal of processing 1 billion dry tons of biomass annually requires focusing the research, at least initially, on the most available, sustainable, and cost-effective feedstocks. Agricultural crop residues have been identified as the most likely high-volume lignocellulosic feedstocks available, with stover and straw being the feedstocks of choice (1). The aim of the present study was to determine the biomechanical properties of wheat and barley straw and corn stover for the purpose of characterizing differences between varieties and their constitutive components. The long-term goal is to provide significant insight into the macroscale harvesting, handling, and processing operations and the microscale plant genetics and fracture behavior (4).

Determining the biomechanical properties of wheat, barley, and corn is challenging for several reasons. First, biologic materials, by their very nature, are complex composite structures whose components are intimately connected. Unlike engineered composite materials, their interconnected behavior makes it difficult to attribute particular mechanical characteristics to any one component. As a result, only bulk material properties can be easily measured, with additional microscale analysis and numerical models needed to discern the attributes of the segregated components. Second, the small size of biologic materials makes the specimens difficult to handle with standard mechanical test equipment. Likewise, their relatively soft tissue structure and unique anatomic arrangement compared to traditionally engineered materials increases the need for highly sensitive and delicate instruments. Finally, the variability of biologic materials requires the testing of several specimens in order to statistically characterize untested parameters such as harvest location, soil composition, stages of maturity, and other variables dictated by nature and not controlled in engineering environments. Thus, results from the mechanic testing of biologic materials have an element of error that is not readily quantifiable.

The testing and measuring of mechanical properties may be considered to be a macroscale operation. Standard testing procedures used on engineered composite structures can be applied to biologic structures to test their performance and determine their material properties under laboratory-applied loads. These loads are within the ranges typically seen in industrial machinery that harvest or process biologic materials and include chopping, grinding, chipping, and billeting (3,5). However, because of the complexity of biologic structures, unique methods must be developed and used to provide sensitive and reliable data within the range of applied loads (6). Mechanical properties determined through laboratory compression, tension, and bend tests can be used as required input to numerical models capable of predicting parameters that affect energy consumption, power requirements, and efficiencies of engineered feedstock-processing systems (7–9). These models will ultimately help optimize machinery design and increase the potential for lowering harvesting, handling, and processing costs.

The physical and mechanical properties of the feedstock are related to the environmental conditions and genetic makeup of the biomass, leading to an additional microscale investigation of the biologic material (10). Data associated with the anatomic structure of the plant material are helpful in interpreting the mechanical property results and determining modes of failure. Thus, it is useful to record and synchronize the visual aspects of the experimental events with load data to determine microscale failure patterns associated with the type of material tested (11). Pre- and posttest observations are also necessary to identify failure mechanisms related to differences in the structure of individual plant components (i.e., vascular bundles, sclerenchyma, parenchyma, and so on) (12–15).

This article presents an approach in which mature (harvested) biomass was collected and tested to determine the modulus of elasticity and ultimate strength for internodal stems of two varieties each of wheat and barley, and four cultivars of corn. A miniature load frame used for an environmental scanning electron microscope was adapted to work with barley and wheat straw, and an Instron load frame was adapted for work with corn (11). The main objective was to develop a database for each variety and determine whether individual varieties could be identified and separated from one another based on differences in biomechanical properties. This database will be used to develop a conceptual use model for testing biomass materials to estimate biomass performance in harvesting, handling, and processing systems. It is recognized that environmental conditions (i.e., temperature, humidity), stages of maturation, matrix composition, and cell matrix configuration are important test parameters to consider (16–22). For purposes of simplicity, this study primarily focuses on matrix composition and cell configuration in mature plant biomass for the determination of biomechanical properties.

Table 1
Plant Variety, Growth Site, and Collection and Testing Dates
for Biomechanical Tests

Variety	Growth site	Collection date	Testing date
Amidon (wheat)	Aberdeen, ID	08/2002	03/2003–03/2004
Westbred 936 (wheat)	Aberdeen, ID	08/2002	03/2003–03/2004
Bowman (barley)	Aberdeen, ID	08/2002	03/2003–03/2004
Fragile Stem 1 (barley)	Aberdeen, ID	08/2002	03/2003–03/2004
Bearclaw 7998 (corn)	Ames, IA	10/2002	04/2004
Dekalb 611 (corn)	Ames, IA	10/2002	04/2004
Garst 8550 (corn)	Ames, IA	10/2002	04/2004
Iowa 550473 (corn)	Ames, IA	10/2002	04/2004

Materials and Methods

Feedstock

Two varieties each of wheat and barley straw and four cultivars of corn stover were selected for use. Table 1 presents the growth site and collection and testing dates for each variety. Selection of the varieties was based on the physical characteristics of straw and stover, primarily those that distinguished one from another.

Westbred 936 is a semidwarf variety of hard red spring wheat with a strong, stiff straw giving it lodging resistance (a plant's tendency not to tipover from external forces). In 2002, it was the top wheat variety grown in southeastern Idaho, and its chemical composition (lignin, hemicellulose, and cellulose content) has also been extensively analyzed at Idaho National Engineering and Environmental Laboratory (INEEL) (23).

Amidon, a standard height hard red spring wheat variety, was chosen because of its moderate resistance to lodging, intermediate level of stem solidness, and medium straw strength. Its semisolid stem distinguishes its cross-sectional composition from that of the more typical hollow-stemmed Westbred 936.

The varieties of wild-type (WT) Bowman and its fragile stem mutant, *fst 1.d* (24), were chosen because of their closely tied genetic makeup. The leaves and stems of the fragile stem mutant plants easily break when physically bent. They are extraordinarily fragile even after maturity. In homozygous lines, straw collapse and/or lodging occurs more frequently compared to the WT Bowman. By contrast, Bowman has good tolerance to late-season lodging and postmaturity straw breakage. It is the parental line used in the introgression of *fst 1.d*.

Each cultivar of corn was chosen based on field standability; apparent strength when handled; and, for logistical purposes, internodal stalk length. The first variety, Bearclaw 7998, is a popcorn cultivar originating in Ohio. It is smaller in stature compared with the other cultivars and has an apparent

weaker stock than most. The next two varieties, Dekalb 611 and Garst 8550, are both commercial cultivars managed in fields at Iowa State University. Dekalb 611 was chosen for its poor standability and long, straight internodal regions. Garst 8550, on the other hand, was chosen for its high standability and long internodal regions. Finally, Iowa 550473 is a parental stiff stalk cultivar originating from Ontario, Canada. It was chosen because of its stiff stalk genetic background and noticeably larger stalk geometry.

The various samples tested were collected during the 2002 cropping season and put into dry, boxed storage until the time of use. The moisture content, though an important physical parameter of biobased materials (5), was not a variable in this study in order to limit the parameters tested and focus on the cellulosic and lignin components of the material.

Testing Methods

Several testing methods—compression, tension, and bend—were used to determine the mechanical characteristics of agricultural residues. These methods were performed with load frames sized to accommodate both wheat and barley stems and corn stover. Video-imaging techniques were used to follow and confirm load test measurements. Pre- and postmortem microscopic analyses helped to identify changes in structural components based on the type of test conducted. Each test provided a range of mechanical data from different parts of the plant and from different varieties of wheat, barley, and corn.

The test results provided two useful quantities: the modulus of elasticity and the ultimate strength of the material. Seven samples from each variety were tested in order to represent their material properties statistically. The mean and standard deviation of these quantities were used to establish similarities and differences among varieties according to their mechanical behavior. Only test data that were complete at the time that this article was written are reported. Therefore, this article contains results from compression, tension, and bend tests of wheat and barley specimens and compression tests of corn specimens.

Selection of Specimens

Test specimens from specific internodal regions were obtained from different plants and different varieties. The testing region chosen for the wheat and barley varieties was the second internode down from the top of the plant, or grain head, as seen in Fig. 1A. Other investigators have used this region, which provides opportunities to compare test results (5,25). Similarly, the corn samples were cut from internodal regions consistent across the four varieties chosen for this study. These regions, however, were referenced from the cob location and not the top of the plant, because the internodes at the cob locations are significantly deformed during growth. Thus, all corn samples were cut from the internodal regions just above and just below the cob locations according to Fig. 1B.

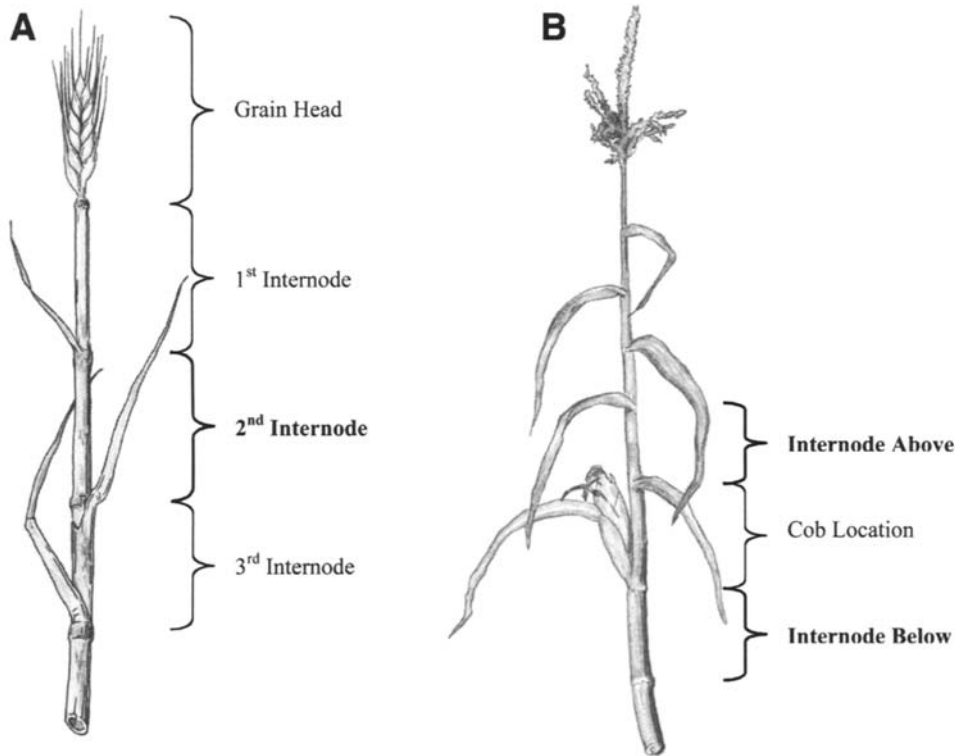


Fig. 1. Illustration of (A) a wheat or barley stem and (B) corn stock. The internodal test regions used are identified in bold.

Bend and Tension Tests

Wheat and barley specimens for the bend and axial tension tests were cut to 76-mm lengths from the center of the second internodal region, providing one specimen per internode per plant. Each specimen was set in the cradle of the bend apparatus with its major axis aligned perpendicularly to the applied load. Data were logged at a rate of two points per second with a load travel rate of 2.4 mm/min. The data included the applied transverse load, the absolute displacement of the load point, and the magnified stem images corresponding to each data point. The image data were used to record surface features and structural failures, and to capture the deflection of the stem needed to calculate the bending modulus for each tested specimen.

Tension test specimens were prepared with special end grips owing to the delicate nature of cereal stems and the waxy coating on the surface. The grips consisted of inner pins that fill the ends of the hollow stems, providing structural support as the jaws were tightened. On the outside of the stem, a self-adhesive heat shrink-wrap was applied to protect the surface of the stem from damage owing to direct contact with the metal jaws.



Fig. 2. Tension test setup for wheat/barley specimens. Shown are the load frame jaws, the end grips, the heat shrink collars, and the extensometer.

Separate collars made from the same heat shrink-wrap were fixed to the stem 1 in. apart and provided attachment points for the knife edges of the extensometer, which was used to measure accurately the strain resulting from the tension loading. Each specimen was clamped within the jaws of the load frame and pulled at a uniform rate of 5 mm/min. Data were logged at a rate of two points per second and included the tension load and the jaw and extensometer displacements. These data were used to create stress-strain curves, from which the slope of the linear portion of the curve was recorded as Young's modulus. Figure 2 is a picture showing the tension test setup.

After testing, both the bend and tension specimens were sectioned through the gage region and a stereo-zoom microscope was used to measure total cross-sectional area, individual component areas, major and minor stem diameters, and wall thickness. These geometric measurements were directly used in the calculation of the area moment of inertia needed for the bending modulus and in the calculations of stress and strain needed for Young's modulus.

Compression Tests

Wheat and barley specimens for the compression tests were taken from the same internodal regions described for the bend and tension tests. Each specimen was cut to an equal height-to-length ratio (1:1) to increase its resistance to buckling. Two samples each were cut from the top and bottom of the internodal region, allowing the potential for differences across internodal stem lengths to be examined. Once cut, the specimens were placed vertically

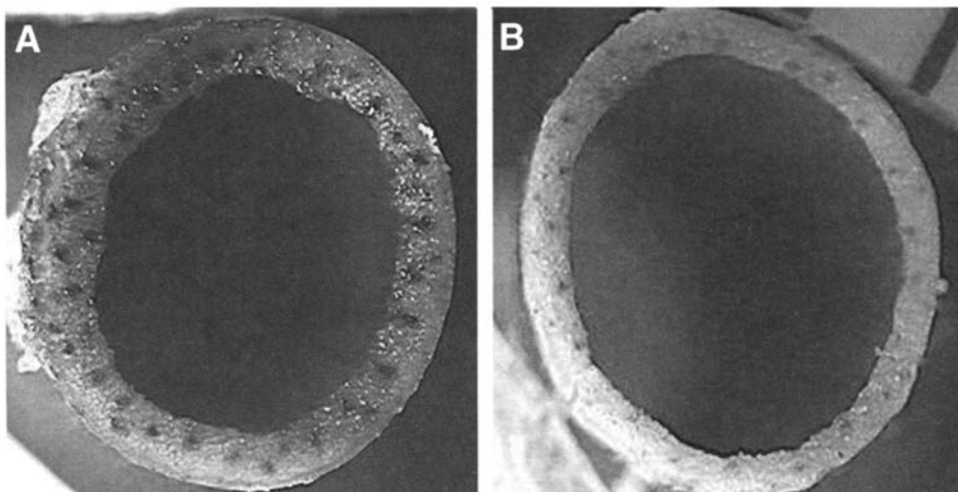


Fig. 3. Cross-sectional images of (A) Westbred wheat and (B) Fragile Stem 1 barley varieties, stained with alcian blue dye (26).

in the load frame and compressed at a rate of 2.4 mm/min. Image data were logged at a rate of two frames per second to record surface features as the specimens failed owing to buckling. Prior to each test, end cross-section images were collected with a stereo-zoom microscope to obtain geometric data required for the calculation of stress-strain curves. The slope of the linear portion of these curves was used to determine the compressive modulus of the specimens.

The compression specimens for corn were prepared in a manner similar to those for wheat and barley, keeping the same length-to-diameter ratio of 1:1. Unlike the wheat and barley specimens, however, the set of seven corn specimens for each variety was cut from the same internodal region, one internode above and below the cob location. This sampling technique provided the means to test variations in the same plant across different stover locations. The test specimens were compressed at a rate of 5 mm/min with load, displacement, and image data collected over the course of the test. These data, along with each specimen's geometric measurements made prior to testing, were used to construct stress-strain curves and determine the compression modulus from the linear portion of these curves.

Results

Figures 3 and 4 show representative stained cross-sectional images of the wheat and barley stems, and corn stover, respectively. These images show details of the sclerenchyma (outer rind or epidermis), parenchyma (inner cells or matrix of the structure), and vascular bundles. These images provide the cross-sectional area data necessary to calculate

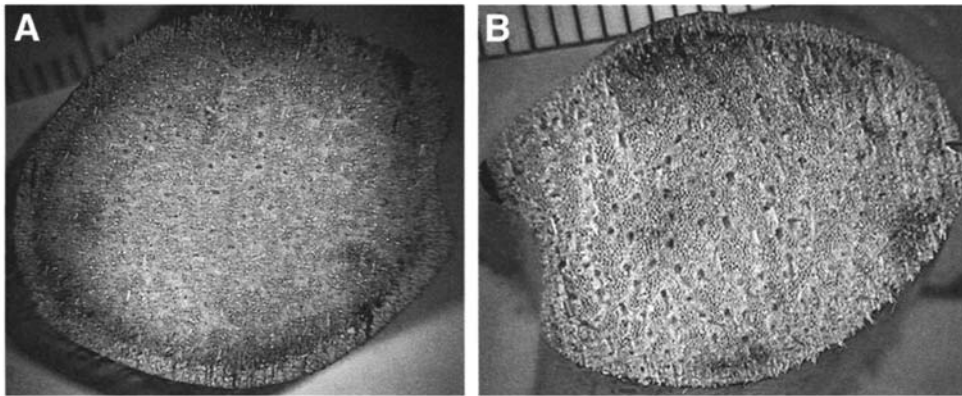


Fig. 4. Cross-sectional images of (A) Iowa 550473 and (B) Dekalb 611 corn varieties.

Table 2
Polymer and Ash Compositions of Wheat, Barley, and Corn Varieties Tested^a

Variety	Cellulose (%)	Hemicellulose (%)	Lignin and extractives (%)	Ash (%)	Unidentified (%)
Amidon (wheat)	38.2	18.7	20.0	5.1	17.9
Westbred 936 (wheat)	39.9	18.6	21.4	6.0	14.1
Bowman (barley)	35.3	16.2	18.2	7.1	23.2
Fragile Stem 1 (barley)	11.1	22.1	16.7	18.3	31.8
Bearclaw 7998 (corn)	32.2	18.1	17.7	5.8	26.1
Dekalb 611 (corn)	35.2	17.6	17.7	3.6	26.0
Garst 8550 (corn)	33.9	13.0	19.1	3.2	30.7
Iowa 550473 (corn)	33.3	17.5	19.5	4.3	25.4

^aCompositions were calculated using a standard quantitative saccharification wet chemistry method (27).

the stress in the stem during testing and to detail the major components of the specimens, which are responsible for the mechanical behavior of each variety.

Table 2 contains details of the chemical composition of each variety tested. The percentages of the four major components of the plant structures (i.e., cellulose, hemicelluloses, lignin, and ash) are reported.

Table 3 provides the modulus of elasticity results from the 3- and 4-point bend, axial compression, and axial tension tests. In all cases, applied load, displacement, and total cross-sectional area measurements were used to calculate the respective modulus. Modulus values for compression and tension were calculated from the slope of the linear portion of the stress-strain curves. For the 3- and 4-point bend tests, moduli were calculated using equations derived from standard beam theory for specimens with