Evaluation of three tractor-guidance methods for parallel swathing at two field speeds

Garris Hudson\*, Robby Shofner†, George Wardlow§, and Donald Johnson‡

ABSTRACT

This study compared the accuracy (mean error and rms error) and precision (standard deviation of error) of three tractor-guidance methods (foam-marker, light-bar, and assisted-steering systems) at two field speeds (5.6 – and 11.5 km/h) for parallel swathing operations. Eighty-four replications of each combination of guidance method and field speed were conducted between 15 October and 22 December 2006 (504 total field passes). The foam-marker system was found to be significantly less accurate [larger mean error (p < .0001) and had a larger rms error (p < .0001)] than either the light-bar or the assisted-steering system. There was no significant difference in mean error (p = .6718) or rms error (p = .8841) by field speed. There was a significant interaction between guidance method and field speed for both mean error (p = .0009) and rms error (p = .003). Mean and rms errors for the foam-marker and the assisted-steering systems increased at higher field speed, while the mean and rms errors for the light-bar system decreased at higher speed. The assisted-steering system had a significantly lower (p = .0164) standard deviation of error (higher precision) than the foam-marker or the light-bar systems. There was no significant difference in the standard deviation of error by field speed (p = .6258) or by the interaction of guidance method and field speed (p = .2748).

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† Robby Shofner is a senior in agricultural education, communication and technology.
§ George Wardlow is a professor of agricultural systems technology management and interim department head of the Department of Agricultural and Extension Education.
‡ Donald Johnson is a professor of agricultural systems technology management in the Department of Agricultural and Extension Education.
INTRODUCTION

Many row crop operations such as tillage, planting, spraying, and spreading require that a tractor and implement make multiple, equal-width parallel swaths through the field. To maximize field efficiency and crop yields, operators must drive accurately to avoid excessive overlaps or gaps in field coverage. Traditionally, visual guidance systems such as mechanical and foam markers have been used as operator guidance aids. With increased machinery working widths, higher field speeds, and extended hours of operation, visual guidance systems have been rendered less effective (Ehsani et al., 2002).

Newer systems are available that use differential global positioning system (DGPS) signals to provide field guidance (Grissio and Alley, 2002). A light-bar (Fig. 1a) provides visual guidance information to the operator, allowing the operator to make manual steering corrections (Trimble Navigation, Ltd., 2005). An assisted-steering system makes these adjustments automatically. One common assisted-steering system (Fig. 1b) incorporates a servo-motor that moves the tractor steering wheel to make these corrections automatically (Trimble Navigation, Ltd., 2005). With assisted-steering systems, the operator only steers the tractor when making turns at the end of the field (Grissio and Alley, 2002).

MEET THE STUDENT-AUTHORS

Garris Hudson

I graduated from Siloam Springs High School, Siloam Springs, Ark., in 2003. After spending my freshman year of college at Northwest Arkansas Community College, Bentonville, Ark., I enrolled at the University of Arkansas in fall 2004, majoring in agricultural education. I was awarded the Alpha Tau Alpha Outstanding Sophomore Award in 2005 and in 2007 my research partner, Robby Shofner, and I won the Gamma Sigma Delta Undergraduate Research Poster Contest. For three years I was actively involved in the University of Arkansas Collegiate FFA Chapter. I graduated in May 2007 with a major in agricultural education and a minor in agricultural systems, technology, and management. I currently live in my home town of Siloam Springs, Ark., with my wife, Serena. I would like to thank Dr. Donald Johnson and Dr. George Wardlow for assisting me and Robby in this research project and for giving me the opportunity to be a part of something as special as this.

Robby Shofner

I am 23 years old and from Bentonville, Ark., where I grew up on a production agriculture farm where we raise purebred and commercial beef cattle. We also managed a 20-acre apple and peach orchard until recently. I graduated from Bentonville High School in 2002 and this spring finished up my undergraduate work at the University of Arkansas majoring in agricultural education. I will be getting married in June and my fiancé and I hope to continue the family farm for many years.
GPS-based guidance varies in accuracy, depending on type of differential correction signal used. Real-time kinematic (RTK) GPS uses a local base station that transmits a correction signal to the RTK GPS unit located on the tractor, resulting in dynamic position accuracies of <2.54 cm (Taylor, 2004). The cost for these systems may exceed $40,000 (Stephens et al., 2005).

Two types of differential GPS (DGPS) are used for guidance. Subscription DGPS uses a commercial signal for differential correction with dynamic accuracies of <10 cm (Taylor, 2004). The annual subscription fee for one common correction signal is approximately $800 - $1500, depending on options (OmniSTAR, 2007). Non-subscription DGPS uses correction signals from the Wide-Area Augmentation System (WAAS) provided at no charge by the US Federal Aviation Administration (Trimble, 2005). WAAS-based DGPS has a dynamic accuracy of <25 cm (Taylor, 2004).

Molin et al. (2002), evaluated the accuracy of a DGPS light-bar guidance system for parallel swathing (5.0-m swath width) at four field speeds between 5.0 and 20.0 km/h. The researchers found that 54% of all errors were ± 0.5 m and that there was no significant difference (p < .05) in mean error by field speed. Karimi et al. (2006) compared seven light-bar guidance systems and found root mean square errors (rms errors) of between 11.1 and 18.6 cm.

There is a paucity of published research evaluating the accuracy and precision of assisted-steering systems. Adamchuk (2007) presented data collected during an extension service field day and determined that RTK, subscription DGPS, and WAAS DGPS assisted-steering systems had mean pass-to-pass errors of 0.76, -3.8, and 24.3 cm, respectively. Adamchuk (2007) indicated that guidance error is affected by GPS error, field conditions, implement tracking, and vehicle dynamics.

The purpose of this study was to determine if there were significant differences (p < .05) in parallel swathing errors by guidance method (foam marker, light bar, or assisted steering), field speed (5.6 – or 11.5 km/h), or the interaction of guidance method and field speed.

The materials and methods section describes the study design, experimental setup, and data collection procedures. A 73.1-m by 73.1-m test plot was surveyed and hub stakes were located at the SW and SE corners to establish the AB baseline for all parallel swathing operations. Six hub stakes were located along this baseline at 13.1-m intervals (Fig. 2a). All measurements were made relative to these six interior stakes. All field passes were made along the east-west axis. Ehsani et al. (2003) and Wu et al. (2005) determined that east-west travel minimizes cross-track errors. Time and weather constraints did not allow including travel axis as an independent variable in the current study.

A John Deere 2355 2WD tractor was equipped with a Trimble AgGPS 132 DGPS receiver, an EZ-Guide light bar (AgLeader Technologies, Ames, Iowa), and an EZ-Steer assisted steering system with a T2 terrain compensation module (AgLeader Technologies, Ames, Iowa). The DGPS receiver was enabled to receive the WAAS correction signal from the Sallisaw, Okla., beacon. The DGPS-based light-bar guidance and assisted-steering systems were configured according to the manufacturer’s instructions (Trimble, 2005; Trimble 2006). A swath width of 3.66 m was set and the light bar was configured so that each LED segment represented 15.2 cm off-line. The assisted-steering system was configured for slightly moderate steering aggressiveness.

A 3-point hitch-mounted toolbar (3.66-m wide) was fitted with a center-mounted spring-tooth shank (5-cm wide) to engage the soil and mark the centerline of tractor travel. The tool bar was also equipped with a foam-marker system with drop tubes located at each end (Fig. 2b).

Eighty-four replications of each combination of guidance method (3 methods) and field speed (2 speeds) were conducted between 15 October and 22 December 2006 (504 total field passes). An AB line was established and 21 parallel swaths were made with the shank engaged with the soil. Right-angle measurements were made between each of the six reference hub stakes and each resulting shank furrow and these distances were recorded. The test plot was dragged after each series of field passes in order to fill the furrows.

For each swath, mean error (mj), root mean square (rmsj) error, and standard deviation of error (stdj) were calculated using the following equations:

\[
m_j = \frac{1}{N} \sum_{i=1}^{N} e_{ij}
\]

\[
\text{rms}_j = \sqrt{\frac{1}{N} \sum_{i=1}^{N} e_{ij}^2}
\]

\[
\text{std}_j = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (e_{ij} - m_j)^2}
\]

Where,

\[\text{std}_j = \text{N} = \text{the number of data points obtained per swath (6)}
\]

\[e_{ij} = \text{the distance from point i to its desired position (j) (error)}\]
Both mean error and rms error are measures of accuracy, while the standard deviation of error is a measure of precision (Ehsani et al., 2002).

The same driver operated the tractor throughout the experiment. This operator could be characterized as a farm-reared college student with previous tractor operating experience, but with no experience in row-crop farming. Prior to training for this study, the operator had no experience with foam-marker, light-bar, or assisted-steering systems.

RESULTS AND DISCUSSION

All mean errors were negative, indicating swath overlap as opposed to swath skips. A 2 X 3 factorial ANOVA indicated mean error for the foam marker was significantly higher (p < .0001) than for the light-bar or the assisted-steering system. There was no significant difference (p = .6718) in mean error by field speed. There was a significant interaction (p = .0009) between guidance method and field speed. The assisted-steering system and the foam-marker were more system accurate at low field speed, while the light-bar guidance system was more accurate at the high field speed (Fig. 3).

Results of a 2 x 3 factorial ANOVA indicated rms error for the foam marker was significantly (p < .0001) higher than for the other two guidance methods. There was no significant difference (p = .8841) in rms error by field speed. There was a significant (p = .003) interaction between guidance method and field speed (Fig. 4).

A 2 x 3 factorial ANOVA indicated that there was a significant (p = .0164) difference in the standard deviation of error, with the assisted-steering system being more precise than the other two systems (Fig. 4). There was no significant difference in precision by field speed (p = .6285) or by the interaction of guidance method and field speed (p = .2748).

Both the light-bar and the assisted-steering systems were more accurate than the foam marker in parallel swathing. The assisted-steering system was more accurate at low field speed, while the light-bar was more accurate at high speed. When using the light-bar at the low field speed, the operator noted a tendency to over-correct; at the high field speed, less time was available for over-correction. When using the assisted-steering system at the high speed, the tractor traveled a greater distance while the automatic steering adjustments were being made, resulting in somewhat larger errors. Additional research should be conducted to determine if increasing steering aggressiveness would increase accuracy at higher speeds.

The assisted-steering system resulted in an overall higher level of precision (as indicated by a lower standard deviation of error) than did the light-bar or the foam-marker guidance systems. This finding was as expected, since automatic systems tend to have a higher degree of repeatability.

Where accurate parallel swathing operations are necessary, farmers should consider use of either a light-bar or an assisted-steering guidance system. Where both accuracy and precision are important, preference should be given to the assisted-steering system.

ACKNOWLEDGMENTS

The authors thank Drs. Johnson and Wardlow of the Department of Agricultural and Extension Education, Dale Bumpers College of Agricultural, Food and Life Sciences. The authors appreciate the assistance of Mr. Vaughn Skinner and Mr. Ron Cox of the Arkansas Agricultural Research and Extension Center for their cooperation in this project. The authors also appreciate the financial assistance for this project provided by the Division of Agriculture, University of Arkansas.

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Fig. 1. Light-bar (left) and assisted-steering (right) guidance systems.

Fig. 2. Field layout (left) and equipment (right) used in evaluation of guidance methods.
Fig. 3. Mean error by guidance method and field speed.

Fig. 4. RMS error by guidance method and field speed.

Fig. 5. STD of error by guidance method and speed. Bars with different letters are significantly different.
A computational model for analysis of uncoupled NO synthase on nitric oxide and superoxide interaction in microcirculation

William J Richardson* and Mahendra Kavdia†

ABSTRACT

Nitric oxide (NO) produced by endothelial cells is a key component for blood-vessel dilation. Dilation is achieved through smooth muscle relaxation as a response to NO transport. Inhibition of this process occurs through the inactivation of NO by reactive oxygen species, especially superoxide (O$_2^-$). NO and superoxide react quickly, forming peroxynitrite (ONOO$^-$. Both superoxide and peroxynitrite apply oxidative stress on vascular tissue. Experimental studies investigating NO interactions are difficult since these reactions occur rapidly and over small distances. This study presents a computational model to describe the interactions of NO, superoxide, and peroxynitrite across an arteriole/venule pair. Based on principles of mass transport, and using knowledge of chemical concentrations and reaction rates, a mathematical model was developed to generate the concentration profiles for NO, O$_2^-$, and ONOO$^-$. We simulated excessive oxidative stress by uncoupled eNOS and determined its effect on NO concentration profiles throughout the region. Based on our understanding of the interactions involved, we predicted 1) increased oxidative stress in the venule decreases NO levels in regions of both the venule and neighboring arteriole, and 2) the amount of NO reduction will vary depending on the location of O$_2^-$ increase. The model demonstrates that different sources of O$_2^-$ have varied effects on NO concentration profiles, and excessive oxidative stress in the venule can impact NO levels in the venule as well as the arteriole. The results provide a more complete description of nitric oxide transfer, which is an important step toward understanding vascular complications in many pathological conditions.

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† Dr. Mahendra Kavdia is an assistant professor in the Department of Biological and Agricultural Engineering and the faculty mentor.
INTRODUCTION

Nitric Oxide (NO) plays a very important role in the human vasculature as a key element in the mechanisms involved in blood-vessel dilation (Bitar et al., 2005; Cook, 2006; Harrison et al., 2006). It is produced by several forms of the enzyme NO synthase (NOS). The most notable form is eNOS, located in the endothelial cells that line the inner surface of blood vessels (Cook, 2006). Shear stress caused by blood flow stimulates eNOS to oxidize the amino acid L-arginine and release NO into the surrounding tissues (Harrison et al., 2006). The target cells are smooth muscle cells where NO is used to convert GTP to cGMP (Cannon, 1998). The increase in cGMP level results in muscle relaxation and thus dilates the vessel.

Inhibition of this process occurs through impaired eNOS production of NO, as well as through inactivation of NO molecules by a number of reactive oxygen species (ROS) (Cook, 2006). The most significant ROS is superoxide (O$_2^-$), which reacts very quickly with NO to form peroxynitrite (ONOO$^-$) (Guzik et al., 2002; Vasquez-Vivar et al., 1998). Both O$_2^-$ and ONOO$^-$ create oxidative stresses in vascular tissues and have a number of harmful effects, such as enzyme inhibition and lipid peroxidation (Kavdia, 2006).

Oxidative stress is exacerbated under pathological conditions such as diabetes, atherosclerosis, and hypertension where O$_2^-$ levels are increased in particular regions of the vasculature, thus decreasing NO availability (Cannon, 1998; Li and Shah, 2004; Wood et al., 2006). Depending upon the condition, the source of ROS varies, and thus the location of oxidative stress also varies. A major source of superoxide during dysfunctional conditions is “uncoupled” eNOS. “Uncoupled” nitric oxide synthase refers to the eNOS enzyme when it is generated with an oxidized form of its normal cofactor, tetrahydrobiopterin (BH$_4$) (Bitar et al., 2005; Harrison et al., 2006). This results in a change in the NO production mechanism as discussed by Li and Shah (2004), and causes eNOS to produce superoxide rather than NO. Under increased oxidative stress during pathophysiological conditions, BH$_4$ is oxidized more readily, resulting in greater concentrations of uncoupled eNOS and thus creating even greater levels of superoxide.

During normal conditions, oxidative stress is kept in balance by antioxidants such as superoxide dismutase (SOD) and carbon dioxide. SOD consumes O$_2^-$, converting it to less harmful oxygen compounds, and CO$_2$ reacts with peroxynitrite, limiting its availability (Kavdia, 2006: Taniyama and Griendling, 2003). A knowledge of interactions between NO, superoxide, and

MEET THE STUDENT-AUTHOR

I graduated in May 2003 from Pulaski Academy in Little Rock, Ark. The following fall, I began my undergraduate studies at the University of Arkansas and was given the Governor’s Distinguished Scholarship, the Chancellor’s Scholarship, and the U.S. Army Corp of Engineers Scholarship. During my time at Arkansas, I have been involved with the University Concert Choir, Tau Beta Pi Honors Engineering Society, and the University Ultimate Frisbee Club Team.

I joined the department of Biological and Agricultural Engineering because of my interest in applying technology and design to problems associated with living systems. My career aim is to work in the medical field performing research and development involving medical devices. I became interested in doing a research project my junior year and began working with Dr. Kavdia on his studies of endothelial function and nitric oxide interactions in the vasculature. In May 2007, I graduated with honors and plan to attend graduate school, pursuing a Ph.D. in biomedical engineering. Conducting undergraduate research and submitting a thesis with Dr. Kavdia has helped to introduce me to academic research and has developed skills that will prove beneficial in my future efforts, both academically and professionally.
peroxynitrite, along with molecules such as SOD and CO2, is crucial to understanding endothelial function and dysfunction. Impact of nearby vessels on NO transport and endothelial function has also been shown. A number of recent studies report that NO produced in the venule can cause dilation of the adjacent arteriole and similarly, NO produced in the arteriole can cause dilation of the paired venule (Guzik et al., 2002; Kavdia, 2006). Increased O2- in the venular wall can impact NO concentrations in both the venule and the neighboring arteriole. Thus, it is important to consider a venule presence in the vicinity of an arteriole when studying NO and O2- interactions.

These reactions occur rapidly and over very small distances, thus testing proves to be difficult. We took a computational approach to study NO and superoxide interactions. The computer model used was based on principles of mass transport. Currently, models exist that consider an arteriole-venule pairing so as to examine NO transport (Kavdia and Popel, 2006), and models exist that consider NO, O2-, and ONOO- interactions so as to examine NO transport (Kavdia, 2006). Our model combines both of these approaches to establish a more complete description of nitric oxide transport in the microvasculature, in the presence of oxidative stress.

MATERIALS AND METHODS

Model geometry. The geometry of our model has been presented previously by Kavdia and Popel (2006) and consists of a tissue containing a paired arteriole and venule as seen in Fig. 1. Each blood vessel has six regions: red blood cell-rich (CR) and red blood cell-free (CF) regions in the lumen, endothelium (E), interstitial space (IS) between the endothelium and smooth muscle layers, smooth muscle (SM), and a nonperfused parenchymal tissue (NPT). These regions are modeled as concentric circles of increasing radii. Vessels are surrounded by a parenchymal tissue (PT) region, assumed to be perfused with capillaries that distinguish it from the NPT. The CR luminal region is assumed to be a homogeneous solution of red blood cells. The PT region represents a homogeneous tissue of capillaries and parenchymal cells (Kavdia, Tsoukias, and Popel, 2002).

As the main production of NO occurs in the endothelial cells by eNOS, NO production is modeled using boundary conditions on the luminal and abluminal surfaces of the endothelial region. Superoxide production in the endothelium is also modeled as surface release incorporated as boundary conditions. In the other regions, O2- generation is included as an overall rate of production. Peroxynitrite is produced only by reaction of NO and O2- and is considered to occur in all regions.

In deriving mass balance of the three species (NO, O2-, and peroxynitrite), the convective transport term was neglected due to the speed of the reactions (Buerk, et al., 2003). Also, concentration profiles have been shown to reach steady state very quickly (Tsoukias et al., 2004). Therefore, mass transport of the species throughout vascular tissues was described using the steady-state mass transport equation (Equation 1). Written in cylindrical coordinates,

\[ D_j \nabla^2 C_j \pm \sum R_{j,i} = 0 \]  

where \( j \) represents the particular molecule of interest; \( C_j \) is concentration; \( D_j \) is diffusivity; and \( R_{j,i} \) stands for production and consumption of the species due to chemical reactions.

Total concentration of peroxynitrite includes concentrations of ONOO- and peroxynitrous acid (ONOONH) (Nalwaya and Deen, 2003). ONOOH is in acid-base equilibrium with ONOO-.

Boundary Conditions. Continuities of NO, O2-, and peroxynitrite mass transport were imposed at each interface between the regions except for the outer edge of the geometry and the surfaces of the endothelium. At the outer edge of the PT, a zero-flux boundary condition was fixed, and at the interfaces with the endothelium, the release of NO and O2- were given by Equations 2a and 2b.

\[ Q_j = D_j \frac{\partial C_{j,\text{CF}}}{\partial r} - D_j \frac{\partial C_{j,\text{EN}}}{\partial r} \]  

\[ Q_j = D_j \frac{\partial C_{j,\text{CF}}}{\partial r} - D_j \frac{\partial C_{j,\text{EN}}}{\partial r} \]  

where \( j \) stands for NO and O2-, and \( Q_j \) represents half of the total release of either species from the endothelium. Both arteriolar and venular endothelial productions were modeled with these equations.

Chemical Reactions. Chemical interactions that were taken into account for the sum of reactions term in Equation 1 vary between regions as each tissue is assumed to be composed of different types of cells. However, all reactions present in the arteriole are considered to be present in similar regions of the venule. In the cell rich region, NO is consumed by hemoglobin contained in red blood cells as a function of the NO concentration, reaction rate with RBC hemoglobin, hemoglobin concentration, and hematocrit. In the smooth mus-
cule region, NO reacts with sGC according to a second-order reaction. In the parenchymal tissue region, NO is consumed and produced by capillaries, according to capillary hematocrit, and capillary volume. Thus O$_2^-$ reacts with NO in the CF, EN, IS, and NPT regions in a second-order reaction in NO concentration.

In all regions, NO reacts with O$_2^-$ to produce peroxynitrite, O$_2^-$ is consumed by SOD, and peroxynitrite is consumed by CO$_2^-$. The reaction-rate expressions for all reactions are presented in Table 1.

Parameter Values. Parameters used in the model are listed along with their values in Table 1. Reasoning for the chosen geometries have been described in detail in previous reports (Kavdia and Popel, 2004; Kavdia and Popel, 2003). A ratio of 0.5 is assumed for the arteriole-to-venule radius values because of reported findings of roughly 0.4-0.5 ratios (Boeghbold, 1996; Nellore and Harris, 2004). Diffusivities of NO, O$_2^-$, and peroxynitrite are assumed to be constant across the geometry and equal 3.3 x 10$^{-5}$, 2.8 x 10$^{-5}$, and 2.6x 10$^{-5}$ cm$^2$/s, respectively (Nalwaya and Deen, 2003; Zacharia and Deen, 2005).

Reaction rate for consumption of NO in the CR region is 1,270 s$^{-1}$ (Kavdia and Popel, 2006). This value is a product of the reaction rate of NO with hemoglobin, heme concentration of 20.3 mM in a single red-blood cell, and a hematocrit of 0.45. Capillary contribution to NO is calculated using a hematocrit of 0.3 and fractional volume of 0.0146 (Ellsworth, Popel, and Pittman, 1988; Kavdia and Popel, 2006). The resulting reaction rate is k$_{cap}$ = 12.4 s$^{-1}$.

NO production is located in the arteriolar and venular endothelia, as well as in the capillary wall and is considered equal (per unit surface area) in these regions. A value of 2.65 x 10$^{-12}$ mol/cm$^2$-s is half of the total NO production rate, and is therefore used for each side of the endothelium (Malinski et al., 1993). The corresponding release rate from the capillary region is 8.6 x 10$^{-7}$ M/s.

Release of superoxide is assumed to be 1.72 x 10$^{-7}$ M/s (20% of NO production) across the whole geometry, excluding the lumen. This is also the surface release rate from endothelial regions. Peroxynitrite equilibrium with peroxynitrous acid is described in the model according to the fraction f, which equals 1/ (1 + 10$^{8k_{per.}}$) with K$_{per}$ = 6.75 (Nalwaya and Deen, 2003). Values for pH in the lumen and the vessel walls are assumed to be 7.4 and 7.0, respectively.

Numerical solution. The system of differential equations generated with equation 1 for NO, O$_2^-$, and ONOO$^-$ was solved using Flex PDE 3.0 software (PDE Solutions, Inc., Antioch, Calif.). We used this software as it has a meshing algorithm that produces a greater amount of elements when the concentration gradient is larger. An adaptive meshing with a relative accuracy of 0.001 was used for the numerical solutions.

Simulations. Profiles for chemical species were generated according to the concentration values along the horizontal center axis of the geometry, and extended 350 µm (100 µm left of the arteriole to 150 µm right of the venule). Along with the base case parameters under normal conditions, the model was used to simulate uncoupled nitric oxide synthase. It has been reported that uncoupled eNOS produces levels of NO approximately 1/3 x the base case scenario and levels of superoxide approximately 3 x the base case (Shinozaki et al., 1999; Vasquez-Vivar et al., 1998). Therefore, to model the impact of eNOS uncoupling, the endothelial surface release rates of NO and O$_2^-$ were multiplied by factors of 1/3 and 3, respectively.

RESULTS AND DISCUSSION

Base case steady state concentration profiles for NO, superoxide, and peroxynitrite. For the base case, we used normal parameters as described in the methods section. Plots of NO, O$_2^-$, and per concentrations for the base case are displayed in figures 2, 3, and 4, respectively. All species reached steady state values within 100 µm of the vessel centers as they proceeded through the vessel walls and into the parenchymal tissue region. These values equal 66.3, 0.084, and 0.88 nM for NO, superoxide, and per concentrations, respectively. Concentration peaks were located in the endothelial regions with a max NO equaling 98.7 nM and occurring in the arteriolar endothelium distal to the venule; max O$_2^-$ equaling 1.6 nM and occurring in the venular endothelium distal to the arteriole; and max per equaling 3.8 nM and occurring in the arteriolar endothelium proximal to the venule. Concentration gradients are steep on either side of endothelial peaks and drastically decrease in the lumen. NO and O$_2^-$ are completely consumed, and per is reduced to 0.014 nM in the venular lumen and 0.52 in the arteriolar lumen.

Uncoupled eNOS impact. We examined the effects of uncoupled nitric oxide synthase on concentration profiles of NO, superoxide, and peroxynitrite by changing surface release parameters at the endothelial regions. Superoxide production was tripled and NO production was multiplied by 1/3. Two simulations were modeled: a) only venular endothelium was considered to be uncoupled, and b) both arteriolar and venular endothelia were considered uncoupled. Resulting concentration profiles are seen in figures 2, 3, and 4 for NO, superoxide, and peroxynitrite, respectively.

Concentrations of NO were drastically affected in both cases. Significant decreases in values occurred at
There was a significant percentage of eNOS that is uncoupled depends upon the percentage of oxidized BH$_4$ due to oxidative stress. Conditions of increased oxidative stress, with little or no effect on NO levels could actually indirectly decrease NO availability in the vasculature by oxidizing BH$_4$ and uncoupling eNOS, which was seen to decrease NO levels in both the venule and arteriole. Taking into account the oxidation of BH$_4$, pathological conditions that raise oxidative stress levels in only the venule could indirectly lower NO levels in both the venule and arteriole through uncoupling of venular eNOS. Including oxidized BH$_4$ percentages in our model could be a significant addition and provide a more complete understanding of these chemical reactions.

In conclusion, numerous studies have experimentally reported changes in interactions of NO, O$_2^-$, ONOO$,\cdot$, SOD, CO$_2$, and uncoupled eNOS during different pathophysiological conditions (Bitar et al., 2005; Cannon 1998; Li and Shah, 2004; Mombouli and Vanhoutte, 1999; Taniyama and Griendling, 2003; Wood et al., 2006). Our model has demonstrated these interactions and provided insight into their possible mechanisms. This understanding is significant to future studies of endothelial and vascular dysfunction, and could potentially lead to improved prevention and treatment of many pathological conditions.

**LITERATURE CITED**


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<td></td>
<td></td>
</tr>
<tr>
<td>Arteriole radius</td>
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<td>µm</td>
<td>Text</td>
</tr>
<tr>
<td>Venule radius</td>
<td>50</td>
<td>µm</td>
<td>Text</td>
</tr>
<tr>
<td>Distance between centers</td>
<td>100</td>
<td>µm</td>
<td>Text</td>
</tr>
<tr>
<td>Art Cell Free thickness</td>
<td>4.5</td>
<td>µm</td>
<td>Rojas et al., 2006</td>
</tr>
<tr>
<td>Ven Cell Free thickness</td>
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<td>µm</td>
<td></td>
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<tr>
<td>Endothelium thickness</td>
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<td>µm</td>
<td>Wood et al., 2006</td>
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<tr>
<td>Interstitial Space thickness</td>
<td>0.5</td>
<td>µm</td>
<td>Kavdia et al., 2002</td>
</tr>
<tr>
<td>Smooth Muscle thickness</td>
<td>6.0</td>
<td>µm</td>
<td>Haas and Duling, 1997</td>
</tr>
<tr>
<td>NPT thickness</td>
<td>5.0</td>
<td>µm</td>
<td>Kavdia and Popel, 2006</td>
</tr>
<tr>
<td><strong>NO reaction rates:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO + O$<em>2$: $k</em>{CO}$</td>
<td>$9.6 \times 10^8$</td>
<td>M$^2$s$^{-1}$</td>
<td>Lewis and Deen, 1994</td>
</tr>
<tr>
<td>NO + O$<em>2$: $k</em>{pop}$</td>
<td>$6.7 \times 10^3$</td>
<td>M$^{-1}$s$^{-1}$</td>
<td>Hule and Padmaja, 1993</td>
</tr>
<tr>
<td>NO + sGC: $k_m$</td>
<td>$5 \times 10^4$</td>
<td>M$^{-1}$s$^{-1}$</td>
<td>Vaughn et al., 1998</td>
</tr>
<tr>
<td>NO + RBC</td>
<td>$1.4 \times 10^5$</td>
<td>M$^{-1}$s$^{-1}$</td>
<td>Carlsen and Comroe, 1958</td>
</tr>
<tr>
<td>NO + RBC in CR: $k_r$</td>
<td>1270</td>
<td>s$^{-1}$</td>
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<td>NO in capillaries: $k_{cap}$</td>
<td>12.4</td>
<td>M$^3$s$^{-1}$</td>
<td>Text</td>
</tr>
</tbody>
</table>
The model area includes the arteriole and venule pair, surrounded by parenchymal tissue (PT) containing capillaries. Both the arteriole and venule have a cell-rich (CR) and cell-free (CF) region referring to the presence or absence of red blood cells in the lumen; endothelium (E); interstitial space (IS) between the endothelium and smooth muscle layers; smooth muscle (SM); and a nonperfused parenchymal tissue (NPT), which contains no capillaries. Figure is taken from Kavdia and Popel (2006).

\[
\text{NO} + \text{ONO}^\cdot \xrightarrow{k_{\text{per}}} 9.1 \times 10^4 \text{ M}^{-1}\text{s}^{-1} \quad \text{Pfeiffer et al., 1997}
\]

\[
\text{O}_2^\cdot + \text{SOD} \xrightarrow{k_{\text{SOD}}} 1.6 \times 10^3 \text{ M}^{-1}\text{s}^{-1} \quad \text{Fridovich, 1995}
\]

\[
\text{ONO}^\cdot + \text{CO}_2 \xrightarrow{k_{\text{CO}_2}} 5.6 \times 10^4 \text{ M}^{-1}\text{s}^{-1} \quad \text{Radi, 1998}
\]
Fig. 2. Effect of uncoupled eNOS on NO profile. To investigate the effect of uncoupled nitric oxide synthase on the species profiles, the parameters for endothelial NO and superoxide production were altered. For the simulation, 1/3 x normal NO release and 3 x normal superoxide release were used in two cases: a) just the venular endothelium, and b) both venular and arteriolar endothelium.

Fig. 3. Effect of uncoupled eNOS on $O_2^-$ profile.
Fig. 4. Effect of uncoupled eNOS on ONOO⁻ profile.
Comparison of instrumental methods for measuring seed hardness of food-grade soybean

Mioko Tamura*, Bo Zhang†, Joyce Berger-Doyle§, and Pengyin Chen‡

ABSTRACT

Seed hardness is an important factor in determining soybean suitability for natto production. There is no established methodology for testing seed texture of soybeans. The objective of this study was to develop an efficient method by examining different instruments and seed parameters that could be potentially used for testing soybean seed hardness. Five food-grade soybean genotypes with different seed sizes were used to determine seed hardness and water-absorption capacity. Water absorption capacity was expressed by swell ratios for seed weight, seed dimension, and volume of water changes before and after soaking. Seed hardness test was conducted by a one-bite method using two food-texture analyzers: a TMS-2000 equipped with shear cell (SC) and a TA-XT2i equipped with either a single blade (SB), a 2-mm probe (PB), a 75-mm cylinder (CY), or a 16-probe pea rigs (PR). The results showed that hardness testing by CY with ten seeds (CV=0.14), SB with 5 seeds (CV=0.11), and SC with 30 g steamed seeds (CV=0.14) produced dependable and consistent results with low coefficient of variance. However, SC may not be practical for early plant selection in a breeding program due to a relatively large sample requirement. Seed size was negatively, whereas swell ratio by weight and volume was positively, correlated with seed hardness, and therefore, can be used as indirect selection indicators for seed hardness.

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§ Joyce Berger-Doyle is a PhD student in the Department of Crop, Soil, and Environmental Sciences.
‡ Pengyin Chen is an associate professor in the Department of Crop, Soil, and Environmental Sciences.
INTRODUCTION

Demand for food-grade soybean has been increasing as more consumers recognize the proven health benefits and nutritional value of processed soybean foods. Soybean isoflavones have been shown to have pharmaceutical functions in preventing cancer, cardiovascular disease, and osteoporosis (Omoni and Aluko, 2005). The proper physical characteristics of food-grade soybeans, such as round seeds with yellow cotyledons, yellow seed coats, and yellow hilium, are desired by consumers for certain soyfoods. Natto is fermented soy product with high, beneficial phytochemical activity, and the natto production utilizes specific types of soybeans including traditional small yellow-seeded, black-seeded, and large-seeded beans. Japan has a large market for food-grade soybeans, and the United States is a major supplier for traditional natto beans every year (Carter et al., 2003).

Seed hardness is an important quality attribute for natto soybean because it affects the water absorption, seed-coat permeability, and overall texture and quality. Seed hardness is affected by calcium content, water absorption, and cookability (Chen, et al. 1993). Calcium content of soybean seeds varies with cultivar (0.19% to 0.52%) but not with different environments (Chen, unpublished data). Water-absorption capacity of soybean seeds is usually measured by swell ratio, which is determined by changes in seed weight, seed dimension, or water volume before and after soaking. Seed-swell ratio is an important trait for determining soyfood quality (Mullin and Xu, 2001). Generally, small-seeded soybeans are preferred for natto manufacturing as they provide for better fermentation than large-seeded soybeans; however, small-seeded soybeans have a tendency to be hard in texture and require more processing time in natto making, which causes undesirable ammonia gas and higher cost of production. To date, very limited research has been conducted to evaluate instrumentation and methodologies for seed-hardness testing. In fact, potential cultivars or product for the natto market are usually examined by professional testers to determine suitability based upon sensory evaluations. At least 0.91 kg of soybean seeds are required in each test in which ten well-trained researchers taste natto products in a four-week period with four replications (Wei, 2004). This method is costly and time-consuming, and it requires making the natto products from soybean seeds. Other methods for evaluating seed texture by instruments include puncture, shear, and compression tests (Bourne, 1972 and 2002; Rhodes, 1972; Okabe, 1979). A puncture test was described as one of the simplest and most commonly used in instrumental measurements for food texture (Bourne, 2002), but it can give rise to large

MEET THE STUDENT-AUTHOR

I am an international student from Saitama, Japan, and currently a junior majoring in crop management with a minor in crop biotechnology. I came to Fayetteville after graduation from technical high school in Japan. I enjoy the activities with the student organizations, such as Grogreen, organic farming club, and ICT (International Culture Team). I am a member of the College Honors Program and have been awarded Harvey A. & Jo York, Eddie Davis, and Hinkle scholarships.

I have been working with the soybean research program since my freshman year and have had opportunities to learn things outside the classroom and a chance to meet many friends. I am interested in organic farming and biotechnology. I hope to have a career in sustainable agricultural development in undeveloped communities. I would like to thank Dr. Pengyin Chen for his support and guidance and members of the research program, especially Dr. Bonnie Zhang, for advice on this project.

Mioko Tamura
materials and methods

test sample preparation.

Five food-grade soybean genotypes, V97-6490, MFL-552, Hutcheson, MFS-591, and Camp, ranging in size from 6.8 to 38.1 g per 100 seeds were grown at the University of Arkansas Agricultural Experimental Station, Fayetteville, Ark., in 2004. All genotypes were subjected to the same growing conditions, and seeds harvested from each genotype were cleaned and sieved to a uniform size. Fifty grams of unbroken and uniform seeds from each genotype were soaked in 250 ml water in heat-resistant plastic boxes for 16 h. Then, seeds were recovered from the soaking water with a sieve and blotted-dried with paper towels. Thereafter, soaked seed samples were pressure-cooked in an autoclave for 20 min at 121.1°C and 1.2 kg/cm². Sub-samples from each genotype were taken, as appropriate, for each type of hard-
ness test.

Seed hardness measurement

Seed hardness of steamed samples was tested with a one-bite method using a TMS Texture System (TMS
-2000, Food Technology Corp. Sterling, Va.) equipped with a 10-blade shear cell and a TA-XT2i food-texture analyzer (Texture Technologies, Scarsdale, N.Y.) equipped with either a single blade, a 2-mm probe, a 75-
mm cylinder, or pea rigs with 16 2-mm probes. The maximum force to puncture, shear, or compress cooked-
seeds in Newtons (N) was determined to represent seed hardness (Song et al., 2003) (Fig. 1). To determine proper sample size for each test method, 20, 30, 40, and 50 g of steamed seeds were used for shear-cell testing; one, five, and ten steamed seeds for cylinder compression testing; one and five steamed seeds for single-blade shear testing; one steamed seed for the 2-mm probe puncture testing; and 16 steamed seeds for pea-rigs puncture testing.

Swell ratio measurement.

Swell ratio was determined by taking dimensions and weight of dry and soaked soybean seeds and comparing volumes of the water absorbed. The seed dimension was measured based on the length, width, and thickness, perpendicular to the hilium, with a digital caliper. Seeds with broken seed-coat and stone seeds (i.e., stone seeds=no water absorption) were discarded from each sample before testing. Seeds were soaked in water for 16 h. Swell ratio by weight was expressed by the ratio of the soaked-seed weight of each genotype to the initial dry weight. Water-absorption capacity of seeds was determined by the absorbed water volume as a ratio to the volume of the seeds.

Statistical analysis.

Hardness test efficiency was assessed by the coefficient of variation (CV), which was calculated by average hardness divided by the standard deviation in each replicated test procedure. The least CV value indicates the least hardness variation among replications with a given testing procedure or sample size. All statistical analysis was performed using SAS (2003). The precision comparison of each hardness-testing method or sample size was evaluated by Fisher’s least significance difference (lsd) test using the general linear model (GLM). The P≤0.05 probability level was used as the statistical-significance threshold when different combinations of replication and sample sizes were compared within and between testing procedures. Pearson’s correlation coefficient (r) was used to determine the relationships between hardness-related traits. The coefficient of determination (R²) of the linear regression model was calculated for each testing procedure.

results and discussion

Swell ratios.

The swell ratio by volume, weight, and dimension was evaluated among all genotypes (Table 1). Cultivar V97-6490, with the largest seeds among all genotypes tested (38.1 g/100 seeds), had the highest swell ratio by volume (2.18), weight (2.42), and dimension (2.78), and its swell ratio by volume and weight was significantly higher than other genotypes. In a previous study, Tachanagaha, with 36.3 g/100 seeds, a Japanese miso cultivar, had a similar swell ratio by weight of 2.84 (Mullin and Xu, 2001). Hutcheson (15.4 g/100 seeds) and MFL-552 (21.8 g/100 seeds) had significantly lower swell ratios by volume (1.82) and by dimension (1.87) respectively, than any other genotypes. Cultivars MFL-552, Hutcheson, MFS-591 (8.7 g/100 seeds), and Camp (6.8 g/100 seeds) all showed lower swell ratios by weight
portionate to sample size for the shear-cell procedure. The one-seed hardness by probe and single blade had similar ranges of 0.8 to 3.4N and 1.8 to 4.3N, respectively, whereas the one-seed hardness by cylinder ranged from 10.5 to 31.1N. These variations were mainly due to equipment design differences that resulted in differences in force and energy needed for compressing (cylinder), slicing and shearing (single-blade and shear-cell), and penetrating (probe and pea-rigs).

**Testing method precision.**

One of the most important characteristics of a reliable method is reproducibility or precision (Guo et al., 2004). Five seeds in SB, ten seeds in cylinder, and 30 g seeds in shear cell were selected to represent single-blade, cylinder, and shear cell due to the low CV (Table 3). Small seed samples tended to cause higher CV, whereas large seed samples reduced the CV for seed hardness measurement. For example, the mean CV of cylinder (0.42) and single blade (0.32) using one seed was significantly higher than that of five or ten seeds (0.20 and 0.15 in cylinder and 0.12 for five seeds in single blade). However, the CVs of shear-cell, using different sample sizes, were similar: 0.15 for 30 g sample; 0.21 for 20 g; 0.18 for 40 g and 50 g samples. In addition, a 20-g sample was not adequate to completely cover the bottom of the shear cell. Therefore, one seed test of cylinder, single blade, or 20-g seeds for shear cell was not proper sampling strategy for a precise hardness test. The probe was not recommended for testing hardness because it yielded one-seed test with a CV as high as 0.32. Similarly, the hardness of cooked, Japanese milled rice using the probe was poorly reproducible as compared to cylinder, shear cell, and single blade (Ohtsubo et al., 1990).

The CV for hardness generated by single blade, cylinder, probe, shear cell, and pea rigs was significantly different (p < 0.05). The CV for hardness using pea rigs and single blade with five seeds was the lowest (0.10 and 0.12) among all the methods using various sizes of seed samples, followed by shear cell with 30-g seeds (0.15) and cylinder with 10 seeds (0.15). However, the pea rigs test was relatively difficult to set up because it also required perfect alignment of 16 individual seeds on the test panel each time, and it required more time to clean the probe. Although shear-cell testing generated low CV, it required relatively large samples, which may not be practical for early plant selection in a breeding program. Therefore, single blade with five seeds and cylinder with ten seeds were highly recommended for testing soybean seed hardness due to the small amount of seeds required and easy setup.

Correlation coefficients (r) for seed hardness measured by the five methods are listed in Table 4. All correlation coefficients were positive except for pea rigs and
probe or single blade. Hardness by cylinder was significantly correlated with hardness by probe, single blade, and shear cell; hardness by shear cell was significantly correlated with hardness by pea rig and single blade. Hardness by single blade was also significantly correlated with hardness by cylinder and probe. Cylinder and single blade had the highest correlation coefficient of 0.81, followed by the correlation between single blade and probe, cylinder and probe, and pea rig and shear cell. Shear cell was relatively weakly correlated with cylinder or single blade, with a correlation coefficient of 0.32. A much higher correlation coefficient (0.94) was found between a single-blade and 10-blade shear cell for the hardness of poultry breast meat (Xiong et al., 2006). The possible reason for the difference between the two studies was that the soybean has different texture as compared to poultry breast meat. In addition, soybean samples loaded in the shear cell consisted of many individual seeds. These correlation coefficients among five methods indicated that cylinder, single blade, and shear cell yielded very similar hardness rankings among soybean genotypes.

**Relationship among seed size, swell ratio, and seed hardness.**

The relationships among seed size, seed-swell ratio, and hardness were modeled using linear regression equations and are shown in Table 5. Seed size and swell ratio by volume, weight, and dimension predicted differently the hardness measured by single blade, cylinder, and shear cell. Seed size and swell ratio by weight were better predictors for seed hardness than swell ratio by volume and seed dimension in single-blade and cylinder procedures. Hardness by single blade was best predicted by the seed size with an $R^2$ of 0.70, followed by the swell ratio by weight with $R^2 = 0.62$ and swell ratio by volume with $R^2 = 0.41$. However, the swell ratio by dimension could hardly predict hardness by single blade due to a very low $R^2$ of 0.01. Hardness by cylinder was best predicted by swell ratio by weight ($R^2 = 0.53$), followed by seed size ($R^2 = 0.45$) and then by swell ratio by volume ($R^2 = 0.30$). Similarly, swell ratio by dimension ($R^2 = 0.01$) cannot be used to predict hardness by cylinder. Neither seed sizes nor swell ratios were good predictors for seed hardness by shear cell because of low $R^2$ values (0.02 to 0.25). Therefore, seed size and swell ratio by weight and volume can be used as indirect selection criteria for hardness without conducting a texture test. Based on the intercept and slope from the regression model, softer seeds tended to be larger and absorb more water, which is in agreement with the results from Taira’s study in 1990.

In summary, cylinder with ten seeds, single blade with five seeds, and shear cell with $30$ g steamed seeds were the most dependable procedures. Cylinder and single-blade probes were more practical for early plant selection in a breeding program than shear-cell probe. Seed size and swell ratio by weight and volume can be used as indirect selection indicators for seed hardness of soybean.

**LITERATURE CITED**


<table>
<thead>
<tr>
<th>Genotype</th>
<th>Seed size (g/100sd)</th>
<th>Swell ratio volume</th>
<th>Swell ratio weight</th>
<th>Swell ratio dimension</th>
<th>PB† 1 seed</th>
<th>SB† 1 seed</th>
<th>5 seeds</th>
<th>LSD</th>
</tr>
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<tbody>
<tr>
<td>V97-6490</td>
<td>38.1</td>
<td>2.2A</td>
<td>2.4A</td>
<td>2.8A</td>
<td>3.4A</td>
<td>4.3bA</td>
<td>27.5aA</td>
<td>1.6</td>
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<td>21.8</td>
<td>2.0B</td>
<td>2.3B</td>
<td>1.9B</td>
<td>1.6B</td>
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<td>15.2aB</td>
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</tr>
<tr>
<td>Hutcheson</td>
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<td>1.8C</td>
<td>2.3C</td>
<td>2.5A</td>
<td>0.8C</td>
<td>2.2bC</td>
<td>8.5aC</td>
<td>0.6</td>
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<tr>
<td>MFS-591</td>
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<td>2.0B</td>
<td>2.3B</td>
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<td>1.4B</td>
<td>1.8bC</td>
<td>8.5aC</td>
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</tr>
<tr>
<td>Camp</td>
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<td>2.0B</td>
<td>2.3B</td>
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<td>0.03</td>
<td>0.43</td>
<td>0.55</td>
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<table>
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<tr>
<th>Genotype</th>
<th>1 seed</th>
<th>5 seeds</th>
<th>10 seeds</th>
<th>LSD</th>
<th>CY†</th>
<th>PR†</th>
<th>SC†</th>
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<tr>
<td>V97-6490</td>
<td>31.1cA</td>
<td>99.1bA</td>
<td>152.5aA</td>
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<td>72.6B</td>
<td>485c</td>
<td>800bA</td>
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<td>48.3bB</td>
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<td>34.1C</td>
<td>348d</td>
<td>483cC</td>
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<td>61.6aBC</td>
<td>4.5</td>
<td>54.2A</td>
<td>491c</td>
<td>670bB</td>
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LSD 15.18 4.74 113.28

†PB, a 2-mm probe; SB, a TA-XT2i equipped with a single blade; CY, a 75-mm cylinder; PR, 16-probe pea rigs; SC, TMS-2000 equipped with a 10-blade shear cell

‡Means with the same lower case letter within a row were not significantly different at p < 0.05.

§Means with the same capital letter within a column were not significantly different at p < 0.05.

<table>
<thead>
<tr>
<th>Traits</th>
<th>VO</th>
<th>WE</th>
<th>Di</th>
</tr>
</thead>
<tbody>
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<td>SS</td>
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<td>-0.51**</td>
<td>0.11</td>
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<tr>
<td>VO</td>
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<td>0.16</td>
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</tr>
<tr>
<td>WE</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* , ** , ***: Significant at P ≤0.05, 0.01, and 0.0001 probability levels, respectively.

SS, seed size; VO, swell ratio by volume; WE, swell ratio by weight; Di, swell ratio by dimension.
Table 3. Coefficient of variation for seed hardness using different testing methods and sample size of five food-grade soybeans.

<table>
<thead>
<tr>
<th>No. of replication</th>
<th>CY†</th>
<th>PB†</th>
<th>SB†</th>
<th>PR†</th>
<th>SC†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 seed</td>
<td>5 seeds</td>
<td>10 seeds</td>
<td>1 seed</td>
<td>5 seeds</td>
</tr>
<tr>
<td>5</td>
<td>0.37Ac</td>
<td>0.20bA</td>
<td>0.14aA</td>
<td>0.35A</td>
<td>0.30bA</td>
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<tr>
<td>10</td>
<td>0.44Ac</td>
<td>0.20Ab</td>
<td>0.15Aa</td>
<td>0.32A</td>
<td>0.34Ab</td>
</tr>
<tr>
<td>15</td>
<td>0.44Ac</td>
<td>0.20Ab</td>
<td>0.15Aa</td>
<td>0.31A</td>
<td>0.31Ab</td>
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<tr>
<td>Mean</td>
<td>0.42</td>
<td>0.20</td>
<td>0.15</td>
<td>0.33</td>
<td>0.32</td>
</tr>
</tbody>
</table>

†CY, a 75-mm cylinder; PB, a 2-mm probe; SB, a TA-ZT2i equipped with a single blade; PR, 16-probe pea rigs; SC, TMS-2000 equipped with a 10-blade shear cell.
‡Means with the same lower case were not significantly different within a row (p < 0.05)
§Means with the same capital letter were not significantly different within a column (p < 0.05)

–, Data not available

Table 4. Correlation among seed hardness of five food-grade soybeans measured by different testing methods.

<table>
<thead>
<tr>
<th>Methods†</th>
<th>CY</th>
<th>PB</th>
<th>PR</th>
<th>SB</th>
<th>SC</th>
</tr>
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<tbody>
<tr>
<td>CY</td>
<td>0.54***</td>
<td>0.09</td>
<td>0.82***</td>
<td>0.32*</td>
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<tr>
<td>PB</td>
<td>-0.09</td>
<td>0.67***</td>
<td>0.15</td>
<td></td>
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<tr>
<td>PR</td>
<td>-0.13</td>
<td>0.53***</td>
<td></td>
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<tr>
<td>SB</td>
<td>0.32*</td>
<td></td>
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</tr>
</tbody>
</table>

†CY, a 75-mm cylinder for 10 seeds data; PB, a 2-mm probe for 5 seeds data; PR, 16-probe pea rigs; SB, a TA-ZT2i equipped with a single blade; SC, TMS-2000 equipped with a multiple blade shear cell for 30 g seeds data.

*, **, ***: Significant at p<0.05, 0.01, and 0.0001 levels, respectively.

Table 5. Regression model statistics for predicting seed hardness from seed physical traits.

<table>
<thead>
<tr>
<th>Traits§</th>
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<th>CY§</th>
<th>SC§</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>0.70</td>
<td>0.45</td>
<td>0.02</td>
</tr>
<tr>
<td>VO</td>
<td>0.41</td>
<td>0.30</td>
<td>0.02</td>
</tr>
<tr>
<td>WT</td>
<td>0.62</td>
<td>0.53</td>
<td>0.06</td>
</tr>
<tr>
<td>DI</td>
<td>0.01</td>
<td>0.01</td>
<td>0.25</td>
</tr>
</tbody>
</table>

§SS, seed size; VO, swell ratio by volume; WE, swell ratio by weight; DI, swell ratio by dimension.
§SB, a TA-ZT2i equipped with a single blade for 5 seeds data; CY, a 75-mm cylinder for 10 seeds data; SC, TMS-2000 equipped with a multiple blade shear cell for 30 g seeds data.
Fig. 1. Five probes used in testing seed hardness of cooked soybean.
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