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DISCOVERY

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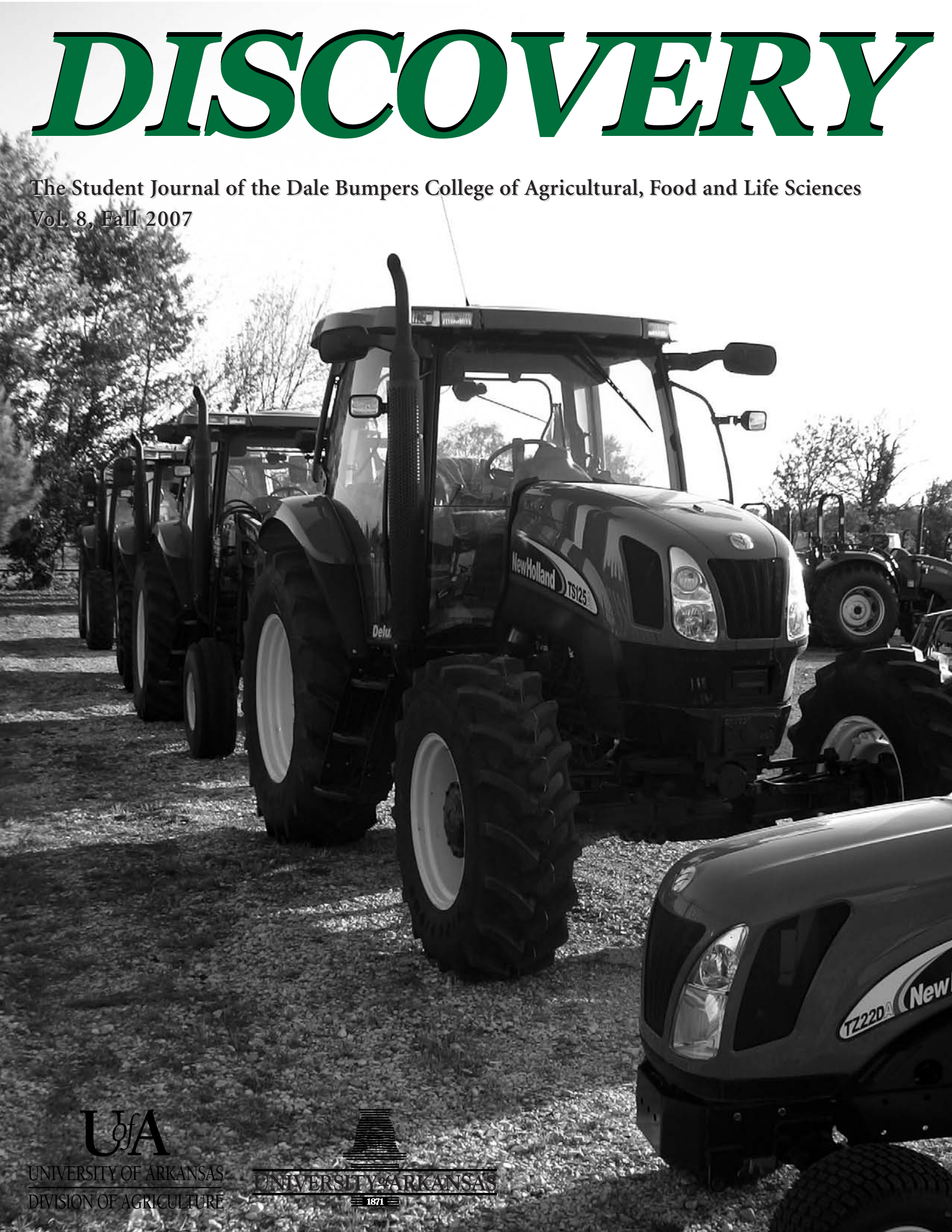

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Contents

Undergraduate Research Articles

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Cover: Compact and larger tractors are adaptable and indispensible to production agriculture, fulfilling central roles in a wide range of on-farm tasks. (Photo by John Crone)

Letter from the Dean

Gregory J. Weidemann

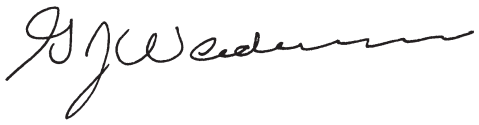
The *Discovery* undergraduate journal is one of the ways that the Dale Bumpers College of Agricultural, Food and Life Sciences encourages students to engage in learning experiences beyond the classroom. The student authors are reporting on the results of research projects they have conducted with faculty mentors.

The *Discovery* journal provides a reporting outlet for our student scholars and scientists. It does not supersede publication elsewhere, but it does provide a forum for students and faculty to share their results and findings in a citable publication.

We encourage student research by awarding undergraduate research grants, including the Carroll Walls Undergraduate Research Fellowship with a stipend of $1,000. Our students also have been very competitive for research and travel grants awarded by the UA Honors College and the Arkansas Department of Higher Education.

Many undergraduate research projects are designed to meet the requirements of an honors thesis in the Bumpers College Honors Program. Our Honors Committee provides a structure that enables our students to enhance their educational experience and provide a very tangible service to society in the process.

We are proud to present these articles as examples of the research accomplishments of our undergraduate students. I heartily congratulate the student authors on their accomplishments and extend thanks to their faculty mentors and to the editors who reviewed their manuscripts.



Gregory J. Weidemann, Dean

Controlling *Listeria monocytogenes* on ready-to-eat poultry products using carboxymethyl-cellulose film coatings containing green tea extract (GTE) combined with nisin and malic acid

Brittany Adams[*], *N.S. Hettiarachchy*[†], *and M.G. Johnson*[§]

ABSTRACT

The ability to control *Listeria monocytogenes* on ready-to-eat poultry products using carboxymethyl-cellulose film coatings containing green tea extract (GTE), malic acid (M), nisin (N), and their combinations was evaluated. The antimicrobials (GTE: 1.0%, nisin: 10,000 IU/g, malic acid: 1.0%) were incorporated alone or in combination into a carboxymethyl cellulose film coating. Pre-inoculated, fully cooked chicken pieces (~1g, 1cm x 1cm x 1cm) were coated with the film solution. The coated chicken pieces were stored at 4°C and the inhibitory activity against *Listeria monocytogenes* was evaluated at 0, 7, 14, 21, and 28 days. The highest inhibitory activity was found in the sample containing GTE, nisin, and malic acid in combination with a reduction of 3.3 log CFU/mL. These data demonstrate that GTE—combined with nisin and malic acid and incorporated into a carboxymethyl-cellulose film coating, multiple-hurdle technology—is effective in inhibiting *L. monocytogenes* growth on fully cooked chicken pieces at 4°C. Research in the area of finding natural antimicrobials to aid in the prevention of food-borne illnesses is necessary to improve safety and shelf life of products such as ready-to-eat meats. This project provides an effective combination of natural anti-microbials to control *L. monocytogenes* in ready-to-eat chicken pieces.

[*] Brittany Adams is a senior majoring in food science.

[†] N. Hettiarachchy, faculty mentor, is a university professor in the Department of Food Science.

[§] M.G. Johnson, a committee member, is a professor in the Department of Food Science.

MEET THE STUDENT-AUTHOR

Brittany Adams

I graduated from Jonesboro High School in 2003 and enrolled at the University of Arkansas in the fall as a food science major. I currently serve as the president of the Food Science Club and am a student member of the Institute of Food Technologists. I am also an active member of the IFT college bowl team for the University of Arkansas. I have received various honors and awards including the Presidential Scholar Award and the John W. White Outstanding Student Award.

I began working for Dr. Hettiarachchy during my freshman year, conducting research in the area of utilizing proteins and anti-microbial plant extracts to inhibit pathogens, which led me to this research project. In 2004, I competed in the Gamma Sigma Delta Undergraduate Research competition and placed 1st in the poster category. I again competed in 2005 and received 1st place in the poster category as well as 2nd place in the Oral Presentation category. I also competed in the Ozark Food Processors Association undergraduate poster competition and received 2nd place for my research in natural anti-microbials for food safety. Once again in 2007, I competed in the Gamma Sigma Delta Oral Presentation and placed 3rd. I plan to enroll in graduate school in the fall in the Department of Food Science and become director of research in Laboratory R&D.

INTRODUCTION

The growing popularity of refrigerated ready-to-eat (RTE) meat products necessitates development of additional pathogen hurdles including chemicals, natural antimicrobials, and novel processing technology to ensure a safe product (Pszczola, 2002). Frequent outbreaks of food-borne illnesses stimulate even greater demand. In 2005, *Listeria monocytogenes* contamination caused 3,086,104 lbs of meat products to be recalled. In 2006, the recall total was 48,346 lbs, while the recall amount for January 2007 was 17,395 lbs. On February 18, 2007, a South Carolina company recalled chicken breast strips due to *Listeria* contamination, with a total of 2.8 million pounds of product being affected (FSIS/USDA, 2007). *L. monocytogenes* can cause serious illness, especially for unborn fetuses and immuno-compromised adults, including the elderly and pregnant women (Lorber, 1990).

Natural antimicrobial agents are effective and inexpensive and can be alternative, practical, and feasible measures to ensure microbial safety of food. Edible films may serve as carriers for such antimicrobial agents as well as act to provide a controlled release of these agents over an extended period of time. Additionally, edible film coatings may prevent moisture loss and maintain freshness (Eswaranandam et al., 2004; Lungu and Johnson, 2005).

An increasing interest in and demand exist for identifying natural antimicrobials, especially of plant origin, that are safe, economical, and effective. The increasing resistance of pathogens to antibiotics further enhances this demand for utilizing plant extracts as alternatives. Plant extracts are a prime source for natural antimicrobials. It is primarily the phenolic compounds in plant extracts, such as green tea extract, that yield antimicrobial and antioxidant activities (Ahn et al., 2004). These phenolic constituents in natural extracts have been shown to be individually effective against bacterial pathogens (Ho et al., 2001; Ahn et al., 2004; Aziz et al., 1998). Epicatechin and catechin phenolic constituents were found to have effective inhibitory activities against pathogenic bacteria (Ho et al., 2001).

Teas are traditionally used in production of beverages

that are recognized for their health benefits, including antioxidant, anti-inflammatory, anti-carcinogenic, platelet-aggregation inhibition, and metal-chelation properties (Yang and Wang, 1993; Bagchi et al., 1998.)

Phenolic compounds in green tea extract (GTE) are believed to be responsible for the compounds' antimicrobial and antioxidant activities (Kim et al., 2004; Ahn et al., 2004). Faculty mentor Hettiarachchy's laboratory has demonstrated the antioxidant activity of tea and grape seed extracts in a model system and in irradiated chicken (Rababah et al., 2004). GTE is commercially available and is used in a variety of food products.

Organic acids, which are naturally present in fruits and vegetables, also act as antimicrobials. Malic acid is a low-cost organic acid naturally found in apples. It has proven affective in killing up to 2.8 Log CFU/mL of *L. monocytogenes* when incorporated in a soy-protein film (Eswaranandam et al., 2004; Hettiarachchy and Eswaranandam, 2007).

Nisin is a bacteriocin produced through the fermentation of *Lactococccus lactis* bacteria. Bacteriocins can be incorporated into food products to control the growth of other microorganisms (Montville and Matthews, 2005). Nisin has been given GRAS (generally recognized as safe) status as a safe biological food preservative (Federal Register, 1988). Janes et al. (2002) demonstrated that nisin is effective in controlling growth of Gram-positive organisms such as *L. monocytogenes*.

MATERIALS AND METHODS

Listeria monocytogenes (v7 serotype 1/2a) was obtained from Dr. Johnson at the Center for Food Safety and Quality research laboratory, University of Arkansas. A commercial sample of nisin, Nisaplin (Alpin & Barrett Ltd., Trowbridge, Wilts., England), was used. Nisin potency was determined by the method of Janes et al. (2002). Commercial green tea extract was obtained from Jarrow Formulas® (Los Angeles, Calif.). Malic acid was purchased from Baker (Phillipsburg, N.J., U.S.A).

Evaluating inhibitory activity against Listeria monocytogenes. A loop of *Listeria monocytogenes* (v7 serotype 1/2 a) was taken from a frozen stock culture stored at –70°C and activated in fresh BHI for 24 h at 37°C in an incubator. For each test, 1.0 mL of the culture was centrifuged (14,000 rpm, 20 min), and the supernatant was decanted. Test solutions in BHI broth containing natural extracts and their combinations were added to the pellet. Nisin (10,000 IU, Franklin et al., 2004), / green tea extract (10 mg/ml) / malic acid (10 mg/ml) and their combinations were added to BHI broth and inoculated with *Listeria monocytogenes* suspensions of 10^6 CFU/ml (colony-forming units per milliliter). Samples were then incubated at 37°C for 24 h and *Listeria* counts determined at 3 h intervals using spread-plate techniques. Results were evaluated to determine the ability of each of the antimicrobial combinations to inhibit *Listeria monocytogenes* in a 24 h time period (Fig. 1).

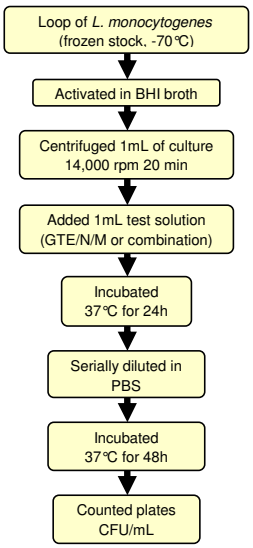


Fig. 1. Flow chart for evaluating inhibitory activity against *Listeria monocytogenes* in broth culture

Preparing films containing antimicrobials. The method for preparing film-forming solutions and films used by Eswaranandam et al. (2004) was followed with slight modifications. The procedure consisted of solubilizing carboxymethyl cellulose (1.75 g) in water (98.25 g), adding glycerol (2.6% w/w) to prevent brittleness, and heating at 90°C for 30 min in a water bath. Nisin (10,000 IU) / green tea extract (10 mg/ml) / malic acid (10 mg/ml) or their combinations were added. The films were cast using the Draw Down instrument from Paul N. Gardner Co., Inc. (Pompano Beach, Fla.) and dried at 50°C and 40% RH for 4 h. The dried films were stored in a dessicator and tested for antilisterial activity (Fig. 2).

Testing antilisterial activity of films. Overnight cultures of *Listeria monocytogenes* (V7 serotype 1/2 a) were inoculated onto 1-cm film discs. Thereafter, 15 µl of the culture was inoculated onto the discs in a Whirl-Pak bag and incubated at room temperature for 1 h. Then 985 µL of phosphate buffer were added to the Whirl-Pak bags and discs were stomached for 2 min. The stomached, inoculated film discs were serially diluted up to 10^4 times using phosphate buffer. They were spread-plated onto *Listeria*-selective agar and incubated for 48 h

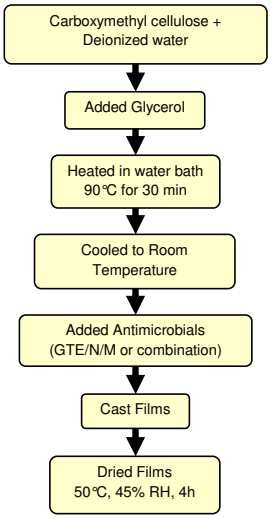


Fig. 2. Flow chart for preparing CMC films

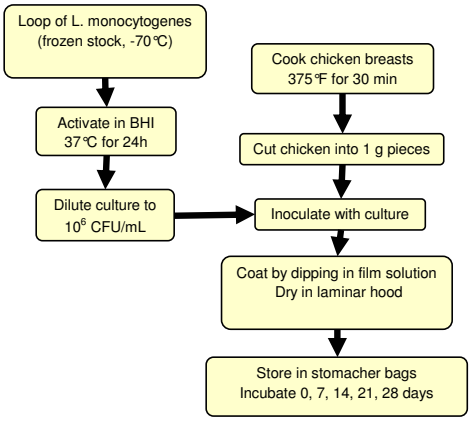


Fig. 4. Flow chart for inoculating and coating chicken pieces

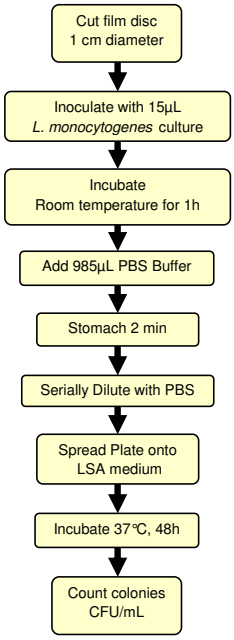


Fig. 3. Flow chart for evaluating anti-listerial activity of CMC films

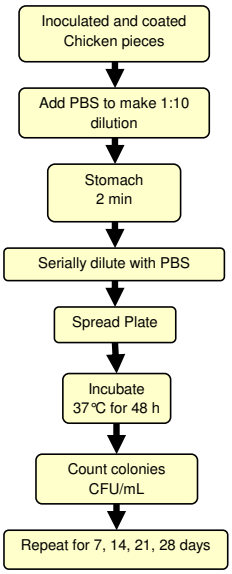


Fig. 5. Flow chart for evaluating anti-listerial activity in meat system

at 37°C. Results were evaluated to determine the log reduction (CFU/mL) given by each antimicrobial film (Fig. 3).

Inoculating with Listeria monocytogenes and coating chicken pieces. Chicken breast products were cooked in a convection oven at 375°F for 30 min and diced to obtain approximately 1-g pieces. These pieces were immersed in 18-h broth cultures of *Listeria monocytogenes* (V7 serotype 1/2 a) containing approximately 10^6 CFU/ml for 30 sec, then removed and allowed to drip free of excess inoculum. This was followed by dipping samples into film-forming solution (carboxymethyl-cellulose) containing green tea extract, nisin, malic acid, or one of their combinations. A total of 21 pieces per d was used (triplicates (3) x 7 treatments including nisin/green tea extract combinations with or without malic acid and a film solution containing no extracts). For controls, 3 pieces per d were used (triplicates). These products were placed into sterile Whirl-Pak bags and refrigerated at 4°C for 28 d and were evaluated every 7 d for inhibitory activity (Fig. 4).

Evaluating antilisterial activity of films containing green tea extract in meat system. Products were removed and evaluated for *Listeria* counts on days 0, 7, 14, 21, and 28. *Listeria* counts were done by placing 9 ml of sterile peptone buffer with 1 g of product (10x dilution) in a Whirl-Pak bag, stomaching, and serially diluting, and the total viable cell counts were determined with the appropriate media (LSA with supplement antibiotic) (EM science, Gibbstown, N.J.) (Fig. 5).

RESULTS AND DISCUSSION

Inhibitory activities of green tea extract, nisin, malic acid, and their combinations in a model system at 37°C.

Fig. 1 shows that *Listeria monocytogenes*, without the addition of antimicrobials (control), grew from an initial level of 6.1 logs CFU/mL to 9.1 logs CFU/mL over 24 h at 37°C in BHI medium. Addition of green tea extract (GTE) or nisin (N) alone allowed *L. monocytogenes* to grow to 9.0 and 8.1 logs CFU/mL, respectively, from the same initial level. Combination of the two (GTE/N) reduced the count to 3.7 logs CFU/mL in 24 h. Malic acid (M) alone reduced the count to non-detectable levels after 9 h while the combinations of M/N and GTE/M reduced them to non-detectable levels after 6 h. The most effective combination was that of GTE/M/N which reduced the counts to non-detectable levels after only 3 h from an initial level of 6.1 logs CFU/mL.

Antilisterial activity of carboxymethyl cellulose films.

Fig. 2 shows log reductions of each antimicrobial combination incorporated in carboxymethyl-cellulose films. Film discs were inoculated with *L. monocytogenes* and incubated at room temperature for 1 h. Control film containing no antimicrobials and film containing only GTE showed very low CFU log reductions of 1.3 and 1.4, respectively. The most effective combination of carboxymethyl-cellulose films was, as before, the combination of GTE/N/M, which gave a log reduction of 4.4 CFU/mL in comparison to the control film.

Inhibitory activity of film solutions on pre-cooked, inoculated, and coated chicken pieces.

1-g pieces of chicken were inoculated with *Listeria monocytogenes* and coated with a carboxymethyl-cellulose coating alone or in combination with the antimicrobials. The chicken pieces were stored at 4°C, inhibitory activity was evaluated on d 0, 7, 14, 21, and 28, and results are displayed in Fig. 3. The control, which was inoculated but not coated, grew from 7.0 logs CFU/mL to 8.2 logs CFU/mL from d 0 to d 28. The sample that was inoculated and coated with only carboxymethyl cellulose grew from 6.8 to 9.1 logs CFU/mL from d 0 to d 28. The combination of GTE/N reduced the counts by d 28 by 2.0 logs CFU/mL when compared to the control with no coating. The combination of GTE/N/M, however, showed the greatest inhibitory activity at 4°C by reducing counts by 3.3 logs CFU/mL by d 28 when also compared to the control with no coating. This value was statistically significant at $p<0.05$.

It is believed that the structure of the phenolic compounds that are present in green tea extract gives it its antimicrobial properties. The ring structure of the phenolic groups is attracted to the lipid membrane of the pathogenic organism while the hydroxyl groups disrupt membrane potential. Addition of nisin and malic acid enhances activity of green tea extract. The unique structure and positive charge of nisin allow it to form pores in the membrane of Gram-positive organisms. These pores allow rapid flow of ions out of the organism and allow malic acid and green tea extract to enter the cell. Once inside, malic acid reduces the internal pH of the cell while green tea extract binds to important enzymes needed for the cell to function. A decrease in log reduction with ready-to-eat chicken could be due to some interactions of the phenolic-active groups with the food components.

This research demonstrates that a combination of antimicrobials incorporated in carboxymethyl-cellulose films is effective in providing an additional hurdle for the growth of *L. monocytogenes* in ready-to-eat chicken products. This may also be applied to a variety of other foods, including fresh fruits and vegetables.

ACKNOWLEDGMENTS

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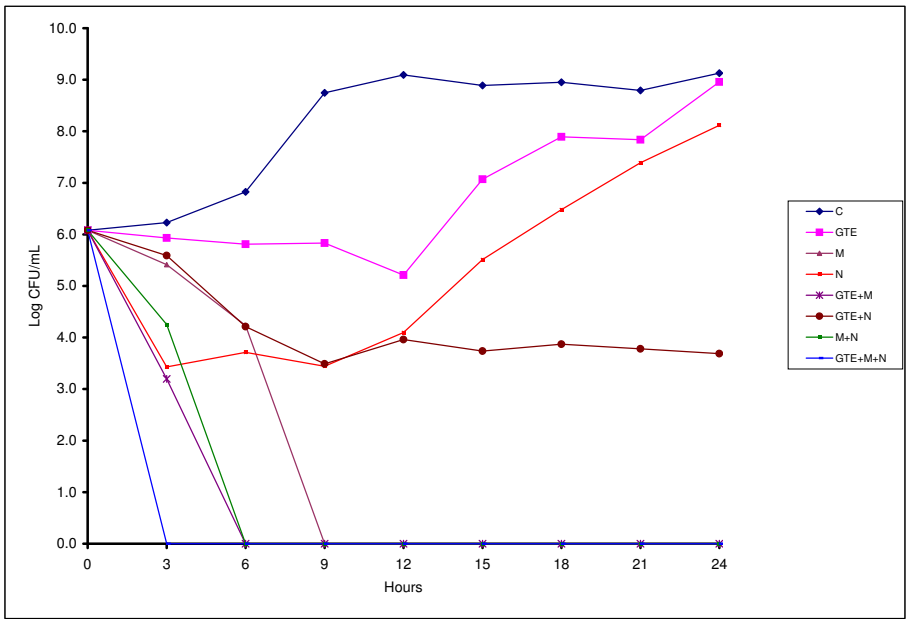
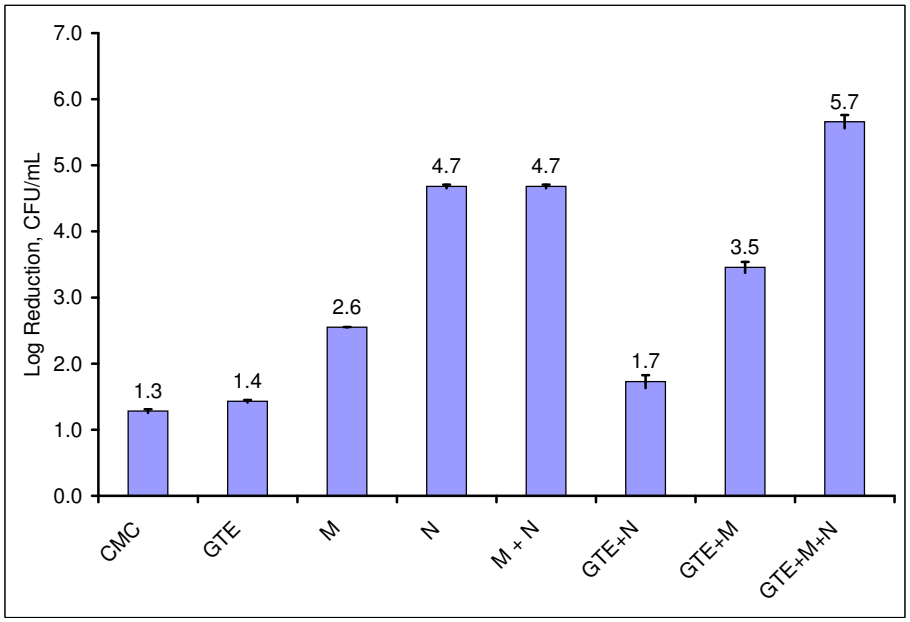


Values are means of three different determinations.
GTE = Green tea extract (1%), N = Nisin (10,000 IU/g), M = Malic acid (1%)

Fig.1. *Listeria monocytogenes* inhibitory activity (log CFU/mL) of green tea extract (GTE), nisin (N), malic acid (M), and their combinations in BHI broth at 37°C for 24 h



Values are means of three determinations.
CMC: Carboxymethyl-cellulose film
GTE: green tea extract (1%), N: nisin (10,000 IU/g), M: malic acid (1%)

Fig. 2. Antilisterial activity of carboxymethyl-cellulose films containing green tea extract (GTE), nisin (N), malic acid (M), and their combinations at 25°C for 1 h.

Treatment	L. monocytogenes Log CFU/g		
	(Days)		
	0	14	28
L.m. Control*	7.0±0.0^{a**}	8.1±0.0^{b}	8.2±0.0^{d}
CMC	6.8±0.0^{ab}	9.1±0.0^{a}	9.1±0.1^{ab}
CMC+M	6.5±0.2^{bcde}	7.1±0.0^{e}	9.0±0.1^{ab}
CMC+M+N	6.5±0.3^{bcd}	5.2±0.1^{h}	8.1±0.4^{d}
CMC+N	6.2±0.1^{de}	7.6±0.1^{d}	8.2±0.1^{d}
CMC+GTE	6.7±0.1^{ab}	9.1±0.0^{a}	9.2±0.0^{a}
CMC+GTE+M	6.7±0.3^{abc}	7.9±0.1^{c}	8.9±0.0^{b}
CMC+GTE+M+N	6.1±0.4^{e}	5.5±0.2^{g}	3.7±0.0^{f}
CMC+GTE+N	6.6±0.1^{abcd}	5.9±0.0^{f}	5.0±0.1^{e}

* Lm control: Inoculation of L. monocytogenes without coating.
CMC: Carboxymethyl-cellulose coating without GTE/Nisin/Malic acid, GTE = green tea extract (1%) N = nisin (10,000 IU/g) M = malic acid (1%)

**All means were measurements of three separate experiments in duplicates. Means within a column followed by same superscript are not significantly different ($p<0.05$)

Fig. 3. *Listeria monocytogenes* inhibitory activity of carboxymethyl-cellulose film (CMC) solutions containing green tea extract (GTE), nisin (N), and malic acid (M) in meat system

The roles, needs, and challenges of Arkansas women in agriculture

Carmen C. Albright[*] *and Jennie S. Popp*[†]

ABSTRACT

Participants of the 2005-2007 Arkansas Women in Agriculture conferences were surveyed for this study to identify recent changes in their roles on and off the farm, the factors important to their success, and the problems they face in their businesses. Respondents were broken into two groups—Farm (women owner-operators of farms, ranches, or agribusinesses) and Non-farm (women working in supporting agricultural industries)—for comparisons and responses were also analyzed across years. Farm women most often reported problems keeping good employees each year, while Non-farm women often reported having problems with being respected as a female business person. For Farm women, the factor most often cited as important to success in their business was being able to pass the business on to family; for Non-farm women it was being able to apply their talents and skills. These results suggest that different types of agricultural women hold different attitudes about business and face different challenges. Results across years suggest that successes and problems may change over time. This marks some of the first research on the roles, challenges, and attitudes of Arkansas' women in agriculture. Based on the results of this research, educational efforts are underway across the state to assist Arkansas' women in agriculture. However, given the small sample of women surveyed, further research is still needed to fully understand the roles, challenges, and attitudes of Arkansas' women in agriculture.

[*] Carmen C. Albright is a spring 2007 Bumpers College graduate with a degree in agribusiness.

[†] Jennie S. Popp, faculty sponsor, is an associate professor in the Department of Agricultural Economics and Agribusiness.

MEET THE STUDENT-AUTHOR

Carmen C. Albright

After my graduation from Ozark High School and retirement from serving as an Arkansas FFA State Officer, I moved to Fayetteville to begin classes at the University of Arkansas, where I was awarded a Chancellor's Scholarship to pursue my studies. At the end of my freshman year, I began working for Dr. Jennie Popp as a research assistant. My first project involved creating a presentation for a grant proposal to organize a conference for Arkansas' women in agriculture. From there, we decided to survey and interview the participants of the conference to learn about their changing roles. After studying abroad in Chengdu, China, for one month, I presented the findings of the survey at the 2005 American Agricultural Economics Association Undergraduate Paper Competition in Providence, Rhode Island, and won third place. I then went on to study abroad in Pau, France, for the fall semester and in Accra, Ghana, West Africa, for the spring semester, with financial support from two Honors College Study Abroad grants and the Richard Locke Scholarship through the Dale Bumpers College. Another survey was distributed at the second Arkansas Women in Agriculture conference, and I won the 2006 American Agricultural Economics Association Undergraduate Paper Competition in Long Beach, California, with findings from the two years' surveys and a comparison of them. Focus-group interviews were held with women throughout the state, and I presented that research combined with the quantitative data at the National Conference for Undergraduate Research in San Rafael, California.

When I was not busy with research and my French and global agricultural, food and life sciences minors, I served as a Dale Bumpers College Ambassador and was active in Sigma Alpha, the professional agricultural sorority. I am now preparing to take some time off in Reno, Nevada, before going to graduate school, but I would eventually like to become a professor.

INTRODUCTION

When the 2002 United States Census of Agriculture was released, a surprising trend was unveiled. Nationally, the number of women principal operators grew about 11% (6% in Arkansas) between 1997 and 2002. Data now show that nationally, 26% (and in Arkansas, 25%) of all women farmers are principal farm operators, meaning they have primary responsibility for day-to-day farm operations. Finally, women are principal operators on 11% of farms nationwide and on 10% of farms in Arkansas (USDA NASS, 2004a; USDA NASS, 2004b).

In addition to these farm women, there are other women who are also important to the agricultural industry. These are women who hold prominent positions in firms that support agriculture, such as agricultural lending institutions, farm input suppliers, veterinary/animal clinics and agricultural processors. Increasing numbers of women in leadership positions both on and off the farm are explained by three factors. First, more women are inheriting these operations due to death, divorce, and illness. Second, women are making voluntary career changes into agriculture. Third, because of the expansion of educational opportunities, more women are choosing college programs that prepare them for important positions in agricultural industries. The number of women receiving agriculture-related degrees has more than doubled since 1993, to 28,801 in 2000 (USDA REEIS, 2000). Even at the University of Arkansas, the number grew by 50% between 1997 and 2005 (University of Arkansas Institutional Research, 2006).

To respond to the increase of women involved in agriculture, researchers need to understand the challenges women face and the goals they hold so as to be successful in their business endeavors. However, success is measured differently by different people. It could mean maximizing income or profit for some (Hoy, Carland and Carland, 1984); it may be non-financial, such as assisting the community or being able to use a particular skill (Buttner and Moore, 1997; Mayasami, Cooper and Valeria, 1999) or it could be some combination of both. Consumer theory in economics is used to show how different goods or services can be combined to achieve some given level of utility or satisfaction (Nicholson, 2004). The level of utility (or in this case, success) received from goods or services (perhaps profits and community assistance) is closely tied to tastes and preferences of the individual. A person can be said to maximize utility subject to a constraint, such as the amount of time in a week. Different agricultural women will have different preferences and constraints and, therefore, can be expected to reach different levels of success or utility from their on-farm or off-farm business/leadership activity.

The importance of the role of women in agriculture has been acknowledged by many states in recent years. Annual conferences for women in dozens of states provide skills to succeed in agricultural production/business, as well as methods to balance demands of family, business, and their communities. The first statewide conference for Arkansas' women in agriculture was held in 2005 with the goal of enriching lives and empowering women in Arkansas in all aspects of agriculture (production, processing, marketing, and retailing) and in their rural communities. Attendees from this and subsequent conferences agreed to participate in research that examined the roles, challenges, and successes of Arkansas' women in agriculture – some of the first research on women in agriculture in the U.S. This paper presents selected results of a three-year study of Arkansas' women in agriculture.

MATERIALS AND METHODS

Surveys of female participants at the Arkansas Women in Agriculture Conference were conducted in 2005, 2006, and 2007. This survey, constructed according to methods described by Salant and Dillman (1994), consisted of two main parts: questions designed specifically for women who owned farms, ranches, or agribusinesses (Farm women), and questions for all female attendees, whether they were farm employees, worked in supporting (e.g., credit, input) industries, retired, or students (Non-farm women). Farm women were given questions about: 1) their operation/business (type, size, location); 2) their role in management; 3) sources of information to assist in that role; and 4) changes in their role and its impact on various decision making and other areas of their lives (use of capital, use of labor, impact on family finances, impact on quality of life for self and others). All female participants—Farm and Non-farm—were asked to respond to questions related to: 1) importance of various characteristics of their work (such as applying their talents to the job, having secure employment, meeting financial needs, balancing work and free time, assisting others in the community, etc.); 2) areas of difficulty in their work (access to credit, networking with others, managing cash flow, marketing products, etc.); 3) farm/community organization involvement, and 4) various demographic characteristics.

For each year, summary statistics were constructed over all questions and all ranges of responses. Responses were then separated into Farm and Non-farm categories. Next, Chi-square and Fisher's Exact tests were used to determine if statistically significant differences existed in the responses. The same tests were used to compare responses between all three years.

RESULTS AND DISCUSSION

A total of 752 female participants at the 2005-2007 Arkansas Women in Agriculture conferences were asked to complete the survey. The response rates were 55% (147 of 269) in 2005; 36% (108 of 300) in 2006; and 49% (89 of 183) in 2007. Only 10% of the 2005 Farm women considered themselves principal operators of their farms or businesses, but 22% in 2006 and 19% in 2007 designated themselves in this role. Between 66% and 75% of Farm women each year responded that they would definitely or probably continue to run the business if something happened to their spouse or business partner. Remaining results focused on the factors important to women in business and challenges they face. Results are presented by year and followed by the comparison between years.

Respondents in 2005

Of the year 2005 respondents, 96 described themselves as farm, ranch, or agribusiness owners (or Farm women). The remaining 51 Non-farm women were employees of ranches and farms, lending institution owner/employees, employees of farm organizations, retirees, and students.

All respondents were asked to indicate whether or not each of 13 factors was important to them in measuring their success in their operation/business. The percent-

ages of Farm and Non-farm women that agreed or strongly agreed with each of the factors are presented in Table 1. Strong significant differences ($p < 0.05$) were found in Farm and Non-farm women's attitudes towards the importance of four factors; a higher percentage of Farm women agreed it was important to be able to pass on the business to family and to provide jobs for the community whereas a higher percentage of Non-farm women agreed it was important to apply talents and skills directly and to be excited about their work.

All respondents were also asked to state their opinion regarding potential problems they face in their work. Results are presented in Table 2. First, on a pure percentage basis, more Farm women reported that problems existed than Non-farm women, with the exception of gaining access to credit. Strong statistical differences were found in attitudes towards three problems; more Farm women reported problems with finding and qualifying for government programs and keeping good employees.

Respondents in 2006

Sixty-nine of the respondents were Farm women; the remaining Non-farm women held jobs and/or memberships in agribusiness, lending institutions, or farm organizations.

Three significant differences existed in Farm and Non-farm women's attitudes regarding important factors in their work life. Farm women felt more strongly that it was important to be able to pass on the business to family, whereas Non-farm women felt more strongly that it was important to feel secure about their employment future and have flexible work hours.

There were also strong significant differences in Farm and Non-farm women's attitudes toward two problems. More Farm women again reported problems with qualifying for government programs and keeping good employees. In addition, significantly higher percentages of Farm women agreed that they face problems of knowing where and how to market their products and keeping financial records.

Respondents in 2007

Of the year 2007 respondents, 45 were Farm women, and 44 were Non-farm women.

Statistical analysis resulted in strong significant differences regarding four factors. Non-farm women were more likely to agree with the importance of applying their talents and skills, trying new ways of doing things, and improving their standard of living. Farm women agreed more with the importance of passing the business on to a family member. Comparisons of the problems faced by each group of women resulted in only one significant difference—more Farm women agreed that they have a problem keeping good employees.

Comparison of all years

Statistical comparison across the three years' of responses yielded very few significant differences (see Table 3). A comparison of Farm women responses across years revealed only two significant differences. First, by 2007 the data suggest that the percentage of Farm women who faced problems being respected as a female businessperson and keeping financial records had fallen from 41% to 33% and from 41% to 25%, respectively. Second, by 2007, data also suggest that the importance of meeting current financial needs in their work success had increased from 82% to 90% for Non-farm women. When comparing all responses across years, only one significant difference was found. Across all respondents, the importance of having flexible work hours in their work decreased between 2005 and 2007. However, as many of the participants in the study varied across years, further research is needed to determine whether these differences exist due to real changes over time or due to changes in participants over time.

Discussion

This research suggests that Farm and Non-farm women share some of the same problems in business as well as the factors they find important to their success. However, because of their diverse backgrounds and dissimilar job responsibilities, their opinions on matters such as the importance of passing on the business to family and having employment security and the problem of keeping good employees are significantly different.

Findings also suggest the importance of conducting research on women in agriculture at different points in time. While the demographic and agricultural information of both years was similar, the needs of these women show different results from the previous year based on the changes of importance on certain factors and the problems they face.

As women become more involved in state and national agriculture, they, like their male counterparts, will experience challenges and, hopefully, success. Currently research and educational efforts are working to address the needs of these women in terms of skill-building and networking, but more research is needed to fully understand the roles, challenges, and goals of different groups of agricultural women at different points in time.

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Table 1. Percentages of women that agreed or strongly agreed with the importance of 13 factors in business.

Factors	2005			2006			2007		
	Percentage of respondents that agree/strongly agree this is important		Probability	Percentage of respondents that agree/strongly agree this is important		Probability	Percentage of respondents that agree/strongly agree this is important		Probability
	Farm	Non-farm		Farm	Non-farm		Farm	Non-farm	
I can apply talents and skills directly	87.4	97.8	0.0451	87.7	94.1	0.2727	78.6	100.0	0.0376
I feel secure in my employment	67.9	88.6	0.1318	72.3	94.1	0.0452	78.6	91.3	0.1565
I can make key decisions	62.8	39.5	0.1209	50.0	32.4	0.1214	57.1	54.6	0.3372
I can meet current financial needs	75.6	81.8	0.8526	75.0	87.9	0.1558	71.4	90.9	0.2899
I can try new ways of doing things	81.2	91.1	0.3663	81.0	97.0	0.1324	65.9	90.9	0.0348
I can participate in environmental conservation programs (ag and non-ag)	81.2	67.4	0.2171	76.2	71.9	0.5363	73.2	68.2	0.5276
I am excited about my work	86.2	89.1	0.0376	84.1	87.9	0.8525	80.5	82.6	0.9800
I have flexible work hours	82.8	80.9	0.4794	69.8	56.3	0.0694	68.3	56.5	0.6481
I can balance my work and free time	64.7	80.9	0.2285	57.1	78.8	0.2872	68.3	82.6	0.5011
I can be involved in my community	83.9	95.7	0.3350	85.7	78.8	0.1275	70.7	78.3	0.6200
I improve my standard of living	77.4	91.3	0.3766	73.0	78.8	0.8190	65.9	95.5	0.0022
I can pass on the business to a family member	79.3	18.6	<0.0001	65.6	16.1	<0.0001	63.4	25.0	0.0233
I can provide jobs for my community	58.8	27.9	0.0094	50.0	42.4	0.5489	42.5	27.3	0.5484

Table 2. Percentages of women that agreed or strongly agreed with problems faced in business.

Factors	2005			2006			2007		
	Percentage of respondents that agree/strongly agree this is a problem		Probability	Percentage of respondents that agree/strongly agree this is a problem		Probability	Percentage of respondents that agree/strongly agree this is a problem		Probability
	Farm	Non-farm		Farm	Non-farm		Farm	Non-farm	
Networking with others	23.5	12.5	0.1224	15.3	27.3	0.3358	20.0	27.3	0.7515
Finding good information about best management practices for my business	26.5	21.4	0.6636	19.7	17.4	0.1258	17.5	13.6	0.8917
Finding information about government programs related to my work	37.4	17.2	0.0452	32.2	22.7	0.7821	30.0	22.7	0.4313
Qualifying for government programs related to my business	33.7	17.9	0.0039	40.7	23.8	0.0423	41.0	21.1	0.3361
Knowing where/how to market my products	30.6	14.3	0.2945	28.8	13.6	0.0691	25.6	20.0	0.9570
Keeping up with environmental regulations regarding my business	40.0	25.0	0.3976	33.9	14.3	0.1778	25.0	30.0	0.7504
Keeping financial records	41.4	14.8	0.1614	33.9	17.4	0.0558	25.0	40.0	0.4705
Finding/affording a good lawyer	42.4	29.6	0.1940	34.5	22.7	0.3942	35.0	15.8	0.2637
Keeping good employees	48.8	25.0	0.0374	51.7	13.6	0.0184	43.6	35.0	0.0708
Handling my cash flow	29.8	19.2	0.5290	29.3	19.0	0.3219	25.0	31.6	0.7525
Gaining access to credit	16.5	22.2	0.1295	10.5	14.3	0.3259	20.0	15.8	0.9273
Completing loan forms and other important paperwork	24.7	18.5	0.3481	15.5	4.8	0.1126	23.1	21.1	0.9892
Being respected in my industry as a female business person	41.2	37.9	0.4902	39.0	36.4	0.2428	32.5	47.6	0.7546

Table 3. Significant differences among 2005, 2006 and 2007 respondents.

Factors	2005	2006	2007	Probability
	Percentage of respondents that agree/strongly agree this is a problem	Percentage of respondents that agree/strongly agree this is a problem	Percentage of respondents that agree/strongly agree this is a problem	
Farm women (problems in business)				
• Keeping financial records	41.4	33.9	25.0	0.0353
• Being respected as a female business person	41.2	39.0	32.5	0.0342
Non-farm women (important factors in business)				
• Meeting current financial needs	81.8	87.9	90.9	0.0992
All respondents (important factors in business)				
• Having flexible work hours	82.0	65.6	64.1	0.0278

Gender and other social effects in people's perceptions of domesticated animals

Clayton W. Bell[*] *and A. Hayden Brown*[†]

ABSTRACT

It is no secret that people possess radically differing opinions and philosophical beliefs regarding domesticated animals. These contradictory perceptions are especially evident when examining people's thoughts regarding the mental capabilities of animals and issues related to animal welfare. To determine whether or not gender and social environments play a role in these various perceptions, a survey was formulated and randomly distributed to 1000 undergraduate students across the University of Arkansas campus. Upon examination of the survey results, some very intriguing correlations became apparent. Of particular interest were the differences between the perceptions of males and females regarding domesticated animals. Women who participated in the survey were significantly more likely to consider pets to be "members of the family" and were twice as likely as men to respond that animals possess a soul. Also, women were less likely to support animal research for medical advancement. These examples illustrate that woman generally hold animals in higher esteem than do men. Another conflicting set of responses came from survey participants who had children versus those who did not have children. According to analysis of participant responses, people with children were drastically less likely to respond that animals were capable of experiencing pain and pleasure. Participants with children were also less prone to consider their pets as "members of the family." Owning pets also had a major impact on the way people viewed domesticated animals. People who owned pets were considerably more likely to respond that animals are capable of experiencing emotions such as love and anger. Also, those participants who own pets were much more apt to respond that domesticated animals are aware of their own existence. Gender and social environments were repeatedly shown to have a considerable influence on people's responses regarding domesticated animals.

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MEET THE STUDENT-AUTHOR

Clayton W. Bell

My name is Clayton Bell and I am a first-year medical student at UAMS (University of Arkansas for Medical Sciences), where I preside on the Honor Council. Before beginning my odyssey at medical school, I spent three wonderful years at the University of Arkansas as an animal science major and a Bumpers College Honors student. While attending the UofA, I was also a member of the Gamma Sigma Delta Honor Society of Agriculture and the Alpha Epsilon Delta Pre-Med Society before obtaining my B.S.A. and graduating with Magna Cum Laude distinction. Fayetteville and the UofA served as an excellent springboard for much of my personal and intellectual growth. Undoubtedly, the solid science background that I attained from the Department of Animal Science has prepared me very well for the rigors of medical school. I would like to give a very special thanks to my Honors College and research mentor, Dr. Hayden Brown, for his support and guidance over the years.

Currently I live with my wife, Heather, and our three dogs on our family farm in El Paso, Ark. We enjoy participating in many outdoor-oriented activities including camping, hiking, and especially mountain biking. We like the mountain biking so much that we travel across the state and compete in the Arkansas Mountain Bike Championship Series with many of our family members and friends. In the future, my goal is to promote health, healing, and happiness by advocating a healthy lifestyle through the art and science of medicine.

INTRODUCTION

Have you ever watched your pet and wondered to yourself, what is he thinking? Unfortunately, there are no indisputable methods to satisfy this inquiry. Instead of hard facts, we are presented only with conflicting hypotheses and philosophical rhetoric. In the end, animals cannot tell us what they are thinking, so we cannot discern the depth of their intellectual aptitudes. This predicament often leads to anthropomorphism, which is the practice of applying human characteristics—such as thought processes—to non-human subjects, such as animals (Serpell, 2003).

Following in the anthropomorphic mode, numerous people advocate that animals possess similar thought patterns to humans. Even history's most famous biologist, Charles Darwin, espoused that there were no characteristics truly unique to man (Povinelli and Bering, 2002). Darwin wrote, "The difference between the mind of the lowest man and that of the highest animal is immense. Nevertheless the difference, great as it is, certainly is one of degree and not of kind" (Darwin, 1871). In addition to proposing that animals possess thinking faculties similar to those of humans, Darwin also viewed domesticated animals as agents capable of experiencing emotions such as disappointment and pleasure. He even went so far as to use those specific terms when describing the behavior of dogs (Wynne, 2004). Undoubtedly for his time, Darwin's perceptions of animals were radical and defiant of his culture's 19th century religious and philosophical dogmas (Povinelli and Bering, 2002).

Over a century later, Darwin's anthropomorphic hypotheses regarding lower animals are still as controversial as ever. Even many of Darwin's contemporaries argued very strongly against the notion that animals could exhibit self-aware thought (Wynne, 2004). In studies where animals supposedly demonstrated rational thought processes, additional in-depth analysis has often contested the studies' results as manifestations of associative learning as an alternative conclusion (Call

and Tomasello, 1999). These controversies may be attributable to the circumstances that different researchers hold different beliefs and opinions, and that those various subjective perceptions influence the way researchers interpret animal behavior and the related research data and observations.

Personal beliefs and opinions also have a major impact on the way we perceive and treat domesticated animals. Unfortunately, personal beliefs and perceptions can put us at odds with some of our peers. Our personal beliefs and opinions can blind us to relative objectivity and at times isolate us from those who think differently than we think. Undoubtedly, people will continue to have diverse opinions about domesticated animals, but learning even some of the reasons why people embrace their specific attitudes and perceptions will help us to better understand and appreciate our peers.

The goal of this research project was not to determine whether or not domesticated animals have the ability to engage in rational thought processes. Instead, the intention was to shed light on why people hold such various beliefs concerning the mental capabilities of lower animals and animal welfare issues. The aim of this research was to explore and perhaps reveal that social environment and demographics can play a major role in what people believe about these topics. Therefore, the objective of this study was to discover correlations between people's demographic and social environments and their opinions and philosophical beliefs pertaining to domesticated animals.

MATERIALS AND METHODS

The research data were drawn from a survey, (see Appendix 1) wherein people were asked for limited personal information and about their social environments, opinions, and philosophical beliefs. Questions were administered in multiple formats. Some questions were answered in a simplistic *yes* or *no* arrangement while other questions provided multiple-choice answers for the survey recipient to choose from, such as which college he/she attended at the University of Arkansas.

To allow for more accurate results, the survey was adapted to Snap Survey Software and was distributed via e-mail to a representative sample population of 1000 University of Arkansas undergraduate students, who were drawn randomly by computers at the University of Arkansas' Survey Research Center. Survey recipients were drawn and categorized by variables such as sex, age, race, undergraduate classification, and to which college at the University of Arkansas the students belonged. The sample population's demographic-variable frequencies were then compared against the entire University of Arkansas undergraduate student body's demographic frequencies to ensure that the sample population was an approximate representation of UA undergrads. The sample population was exceptionally well-drawn, especially with regard to the male-female ratio and the frequency of students from each respective college within the University of Arkansas.

After obtaining an approximate sample population, the survey was distributed to the 1000 survey recipients simultaneously through a Microsoft Outlook e-mail. As the survey participants completed and submitted their surveys, the results were immediately returned to a secure data collection account. After one week, 129 sets of survey results were collected, then passed on to a password-protected SPSS Survey Software account. To ensure utmost privacy for respondents, survey results were transmitted without any form of student identification. Within the SPSS Survey Software, survey responses were collaborated, categorized, analyzed, and interpreted. Bar graphs and pie charts were utilized to illustrate demographic and social effects relative to people's perceptions of domesticated animals. Pearson Correlation Analysis was employed to identify significant correlations between people's demographics, social environments, and perceptions of domesticated animals. The Pearson Correlation Analysis identified significant positive and inverse correlations at the 0.05 level ($P<0.05$) and the 0.01 level ($P<0.01$).

RESULTS AND DISCUSSION

Whether a person was male or female was demonstrated to have a major effect on that person's perceptions of domesticated animals. Males were much more likely to support animal research for the benefit of medical advancement (.290, $P<0.01$), and males were significantly more likely to be hunters when compared to females (.354, $P<0.01$). This strong correlation between surveyed males and hunting reinforces the male's traditional role as the predominant hunter throughout human history (Molnar, 2006). On the other hand, women were significantly more likely to consider their pets to be "members of the family" (.195, $P<0.05$), and women were twice as likely as men to respond that animals possess a soul (.174, $P<0.05$) (Fig. 1). These results strengthened the idea that women are more likely than men to develop a strong connection with domesticated animals. According to Jahme et al 2001, women are able to feel such strong connections with animals because, "Women intuitively understand much of what the animals are feeling" (Jahme et al, 2001).

Having children also had profound effects on people's perceptions of domesticated animals. Participants with children were significantly more likely to support animal research compared to participants without children (.192, P<0.05). Also, people with children, when evaluated against people without children, were less likely to respond that animals were capable of experiencing pain and pleasure (.174, P<0.05). Furthermore, parents were far less likely to respond that animals have souls when compared to non-parents (.182, P<0.05), and although the correlation was not significant at the 0.05 level, people with children were much less likely to consider their pets to be "members of the family." It appears from this survey that people without children tend to hold animals in higher esteem. One probable rationale is that a parent's admiration for animals begins to wane as they develop a prioritized appreciation for and bond with their children.

Owning pets also appears to have a major impact on the way people view domesticated animals. As one might imagine, survey participants who owned pets were far more likely to consider pets to be "members of the family" when compared to participants who did not own pets (.307, P<0.01) (Fig. 2). People with pets also appeared significantly more likely to respond that animals are aware of their own existence when evaluated against respondents with no pets (.186, P<0.05). Furthermore, respondents with pets were much more likely to respond that animals are capable of experiencing emotions such as love and anger (.201, P<0.05).

Those participants who responded that pets could experience emotions such as love and anger were significantly more likely to respond that animals also had the ability to experience pain and pleasure (.269, P<0.01). Individuals who responded that pets could experience emotions were also significantly more likely to respond that animals were aware of their own existence when evaluated against respondents who did not indicate that pets could experience emotions (.327, P<0.01). Similarly, participants who responded that animals were aware of their own existence also believed that animals could experience pain and pleasure more often than survey participants who disputed the notion that animals were aware of their own existence (.184, P<0.05). From these three viewpoints, a triad of perception emerges (Fig. 3). It becomes evident that a person who selects one of these three perceptions will also be significantly more likely to embrace the other two perceptions as well.

Nearly all of the survey participants agreed that animals could experience pain and pleasure (96.12%). Most participants also responded that animals could experience emotions (78.91%), and many participants responded that animals were aware of their own existence (61.24%). However, there was much more controversy over the idea that animals possess souls (Fig. 4). The group in strongest opposition to the perception that animals have souls were the supporters of animal research for the benefit of medical advancement (.293, P<0.01). Participants who hunt also strongly opposed the notion that animals possess souls (.287, P<0.01). This makes logical sense, because people who responded that animals have souls were far less likely to utilize animals for food or knowledge. Those students who responded that animals have souls also predominantly responded that animals have emotions (.240, P<0.01). Significant correlations also existed between participants who responded that animals have souls and participants who responded that animals are aware of their own existence (.187, P<0.05).

Participant responses also provided some interesting insights into the realms of human spirituality and science. Not surprisingly, participants who responded that animals have souls also responded that people have souls (.431, P<0.01). Students who responded that they themselves have souls were extremely likely to indicate belief in a divine creator (.653, P<0.01). This positive correlation between the perceptions of people having souls and a divine creator was the strongest correlation found throughout the entire research project, yet these perceptions appeared to be inversely related to people's opinions about Darwin's theory of evolution. Participants who agreed with evolution were far less likely to respond that people possess souls (.285, P<0.01), and the respondents who support the theory of evolution were even less likely to believe in a divine creator (.306, P<0.01). According to the survey participants' responses, it appears that some of the sampled group believe in a divine creator and people having souls and some believe in Darwin's theory of evolution, but few responded with both alternatives.

In conclusion, demographic and social variables most likely have an effect on the way people perceive domesticated animals. Furthermore, these effects can be isolated and analyzed to discover predisposed perceptual tendencies that a person might have. By analyzing a person's demographics and social environment, we will have a better opportunity to understand and appreciate another's beliefs. When we develop a greater comprehension and respect for another person's beliefs, we give ourselves an opportunity to connect with that individual on a more personal, intellectual, and intricate level. This could be beneficial in the classroom, in business, and even among friends and colleagues.

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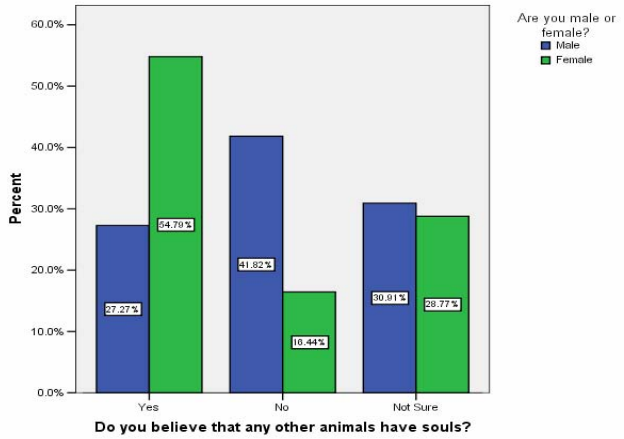


Fig. 1. According to responses from survey participants, women were twice as likely as men to believe that animals possess souls.

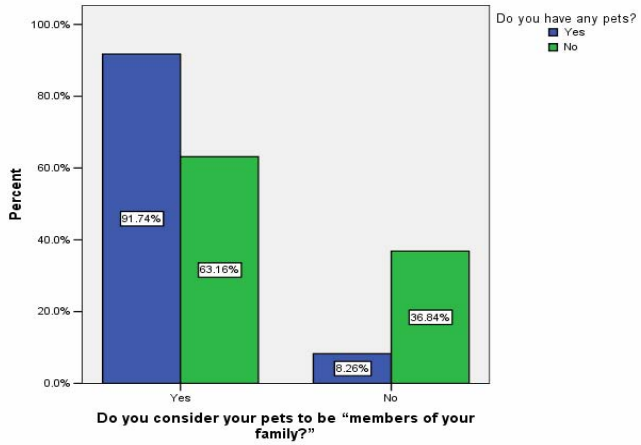


Fig. 2. The effect of having pets on people's perceptions of pets as "family members." People with pets almost always consider pets to be "members of the family," while people without pets are much less likely to perceive pets as worthy of "family member" status.

Correlations

		Do you believe animals are aware of their own existence?	Do you believe domesticated animals are capable of experi...	Do you believe domesticated animals have emotions such as...
Do you believe animals are aware of their own existence?	Pearson Correlation	1	.184*	.327**
	Sig. (2-tailed)		.037	.000
	N	129	129	128
Do you believe domesticated animals are capable of experi...	Pearson Correlation	.184*	1	.269**
	Sig. (2-tailed)	.037		.002
	N	129	129	128
Do you believe domesticated animals have emotions such as...	Pearson Correlation	.327**	.269**	1
	Sig. (2-tailed)	.000	.002	
	N	128	128	128

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Fig. 3. These three sets of beliefs form a triad of perception. If a person embraces one of these perceptions, it is highly likely that he/she will also embrace the other two beliefs.

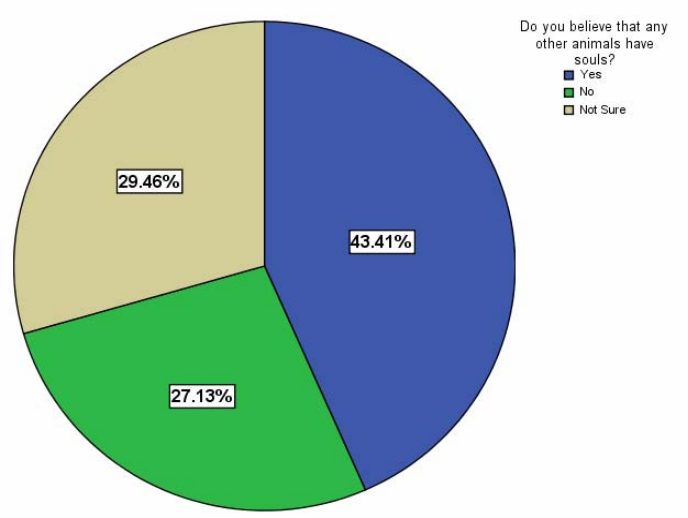


Fig. 4. Participants' responses on whether or not animals have souls proved to be highly controversial.

Appendix 1

Demographic and Social Effects on People's Perceptions of Domesticated Animals

My name is Clayton Bell, and I am an Animal Science Honor Student at the University of Arkansas. I have formulated this survey so that I may find the correlations between people's demographic and social backgrounds with respect to their perceptions of domesticated animals. In essence, this thesis project will enable me to objectively identify which environmental influences predispose an individual to hold certain beliefs about domesticated animals. Participation in this survey should only be completed on a voluntary basis. Refusal to participate will not result in any penalties or loss of benefits whatsoever. If you choose to participate, your survey shall be kept completely confidential through the security of the SNAP Survey Software. Once your survey has been completed you will be freed of all ties to this research project. If you have any questions please feel free to contact me through e-mail at cwbell@uark.edu <mailto:cwbell@uark.edu>.

Q1 **What is your favorite animal?**

Q2 **Do you have any pets?**

Yes

No

If so which species?

Q3 **Do you consider your pets to be "members of your family?"**

--Click Here--

Q4 **Did you grow up with animals?**

--Click Here--

Q5 **Have you ever owned or worked with livestock?**

--Click Here--

Q6 **Do you eat meat?**

--Click Here--

Q7 **Do you hunt?**

Yes

No

If so which species?

Q8 **Do you support animal research for the benefit of medical advancement?**

--Click Here--

Q9 **Do you believe domesticated animals are capable of experiencing pain and pleasure?**

--Click Here--

Q10 **Do you believe domesticated animals have emotions such as love and anger?**

--Click Here--

Q11 **Do you believe domesticated animals communicate with each other?**

--Click Here--

Q12 **Do you believe animals are aware of their own existence?**

--Click Here--

Q13 **Do you believe that domesticated animals engage in rational thinking?**

--Click Here--

Q14 **Do you believe people engage in rational thinking?**

--Click Here--

Q15 **Do you believe that people have souls?**

--Click Here--

Q16 **Do you believe that any other animals have souls?**

--Click Here--

Q17 **Please list which animals.**

Q18 **Regardless of religious preference or lack thereof; do you believe in a divine creator?**

--Click Here--

Q19 **What is your religious affiliation?**

Christian *Buddhist*

Jewish *None*

Muslim *Not Sure*

Other (Please specify)

Q20 **Do you believe in evolution?**

--Click Here--

Q21 **Do you believe that other life forms (animals, plants, etc.) exist for our benefit?**

--Click Here--

Q22 **Do you believe that we exist for the benefit of other life forms (animals, plants, etc.)?**

--Click Here--

Q23 **Do you believe all life forms exist to benefit each other?**

--Click Here--

Q24 **Are you male or female?**

--Click Here--

Q25 **What is your age?**

Q26 **What is your race?**

--Click Here--

Q27 **What college at the UA do you reside in?**

Dale Bumpers College of Agriculture, Food, and Life Sciences

School of Architecture

J. William Fulbright College of Arts and Sciences

Sam M. Walton College of Business

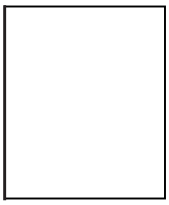
College of Education and Health Professions

College of Engineering

Honors College

Q28 **What is your current class standing?**

--Click Here--



Q29 **Do you have any children?**

--Click Here--

Design of a bioreactor to study the role of red blood cells in the transport of nitric oxide in the microcirculation

Nupura Bhise[*] *and Mahendra Kavdia*[†]

ABSTRACT

Nitric oxide (NO) plays an important role in physiological functions like vasodilation, neurotransmission, and inhibition of platelet aggregation. The endothelium-derived NO diffuses into the vascular lumen where it interacts with flowing blood as well as the smooth muscles where it modulates vascular tone. However, uncertainty exists on how NO escapes the rapid scavenging by hemoglobin (Hb) and reaches smooth muscles. Several proposed hypotheses include 1) a reduced reaction rate of NO with Hb contained inside red blood cells (RBCs) and 2) NO preservation in the bound form of s-nitrosohemoglobin or nitrite. The mechanism and magnitude of reduction of NO reaction rate with Hb contained inside RBCs are not well established. In this study, an in vitro experimental system was designed to expose stirred RBC suspension to physiologically relevant NO flux. NO-RBC interactions were studied by measuring the reaction products, nitrite and total NO_x, using chemiluminescence method. We studied the effect of increasing hematocrit from 5% to 45% on NO-RBC interaction under oxygenated condition. Results show that the system maintained a steady state in the bioreactor and could be easily modified to control NO delivery flux. An increase in product concentration was observed by increasing the hematocrit from 5% to 45%. The study is clinically important as the understanding of molecular interaction of NO with Hb in RBCs and mode of NO transport in microcirculation may provide therapeutic opportunities in the biomedical field in areas as diverse as sickle cell anemia, septic shock, hypoxic pulmonary vasoconstriction, and blood substitutes.

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

INTRODUCTION

Nitric oxide (NO) mediates significant vascular endothelium functions, including vasodilation, blood pressure regulation, inhibition of platelet aggregation, neurotransmission, and immunological response (Moncada et al., 1991). In the microcirculation, NO is synthesized via enzymatic oxidation of amino acid L-arginine to L-citrulline by endothelial NO synthase enzyme. Endothelium-derived NO diffuses into the vascular lumen where it interacts with flowing blood as well as smooth muscles where it activates soluble guanylate cyclase and modulates vascular tone. Discovery of NO as endothelium-derived relaxation factor led to the awarding of a 1998 Nobel Prize in physiology to investigators F. Murad, R.F. Furchgott, and L.J. Ignarro (Gow et al., 1999). Subsequently many scientific investigators have been involved in studying a possible role for red blood cells (RBCs) in transport of NO and its vascular effect in facilitating flow of blood through the microcirculation.

Homeostasis of NO is a balance between its synthesis and consumption. In the lumen, NO is either oxidized by oxygen (O_2) resulting in nitrite (NO_2^-) formation, or is taken up by red blood cells, which contain high concentrations of hemoglobin (Hb), an effective NO scavenger. Two important reactions of Hb and NO considered to be relevant are (a) the oxidation, in which NO reacts with oxyHb ($HbFe^{2+}O_2$) to form nitrate and metHb ($HbFe^{3+}$), and (b) the addition reaction, in which deoxyHb ($HbFe^{2+}$) binds to NO to form $HbFe^{2+}NO$ (HbNO). Due to this high reactivity of NO with Hb and other species, NO may be consumed before it travels from its synthesis site (endothelium) to target site (smooth muscle). Earlier studies thus assumed that the only effect of RBCs on the bioactivity of NO was to scavenge and inactivate NO, thus limiting its ability for

MEET THE STUDENT-AUTHOR

I am an international student and graduated in spring 2007 with a biological engineering major. I completed high school in Mumbai, India, and joined the University of Arkansas in fall 2003. Beginning in November 2003, I worked as an undergraduate assistant for Dr. Mahendra Kavdia and helped him set up his Vascular Biotransport Laboratory; because of this opportunity, I fulfilled my professional aspiration to work in a biomedical laboratory setting under his guidance. I have been involved in research studying the in-vivo interaction of red-blood cells with nitric oxide. My work, which was funded thrice by the U of A Honors College Undergraduate Research Grant, has yielded four conference presentations, and I plan to submit a paper for professional journal publication.

Nupura Bhise

Since my freshman year at the university, I have been involved each year with on-campus professional and community organizations. I am currently serving as the president of the Biological Engineering Students Club and served as its treasurer in my junior year. I was also the president of the Golden Key International Honour Society and served as a mentor for the Society for Women Engineers. I have been awarded many prestigious academic scholarships and honors including the Outstanding Senior Scholar award (Arkansas Academy of Biological and Agricultural Engineers), and First-Ranked Senior Scholar, U of A. I have been accepted for the PhD program in biomedical engineering at the Johns Hopkins University and plan to begin in fall 2007.

I would like to thank Dr. Kavdia for his support and guidance in my research as well as academic career at the U of A.

vasodilation (Nagababu et al., 2003). However, the NO bioactivity is preserved in the blood under normal physiological conditions. Scientists trying to investigate how NO escapes rapid scavenging by Hb have proposed various hypotheses including (a) an erythrocyte-free layer in the lumen adjacent to the endothelium that provides an effective diffusional barrier; (b) about a 500- to 1000-times lower effective reaction rate of NO with Hb contained in RBC than that with free Hb, $k \approx 10^7\ M^{-1}\ s^{-1}$ (Huang et al., 2001; Liu et al., 2002); and (c) NO preservation in the bound form of s-nitrosohemoglobin (sNOHb) (Gow et al., 1999; Pawloski et al., 2001; McMahon et al., 2002).

In spite of these studies, individual roles of these proposed factors for regulation of vascular functions remain largely unknown. Uncertainty also exists on the mechanism responsible for NO preservation and the magnitude of effective reaction rate of NO with RBCs and its relationship with hematocrit and flow. To overcome the technical difficulties of in vivo determination of the local availability of NO and study this relationship, we designed an in vitro constantly stirred experimental setup that simulates in vivo conditions of NO synthesis and consumption. We delivered NO at a constant flux to a constantly stirred RBC solution in the bioreactor and measured the products of RBC-NO interaction: nitrite (NO_2^-) and total NO-species (NO_x), using a gas-phase chemiluminescence method.

The in vitro studies provide a critical step in quantifying NO uptake by varying the hematocrit concentration from 5% to 45% in oxygenated condition and extend our understanding of the functional role of NO in the microcirculation. This knowledge is critical to development of hemoglobin-based oxygen carriers (HBOCs) that are under development as an alternative to RBC transfusion because of the potential advantages of unlimited supply, no cross-matching requirement, chemical purity and prolonged storage (Winslow, 2000). The understanding of molecular interaction of NO with Hb and NO transport is clinically important and may provide therapeutic opportunities in areas as diverse as sickle cell anemia, septic shock, NO inhalation, hypoxic pulmonary vasoconstriction, and blood substitutes (HBOCs).

MATERIALS AND METHODS

Materials.

All the chemicals—sodium iodide (NaI), sodium nitrate (NaNO3), sodium nitrite (NaNO2), phosphate saline buffer (PBS), EDTA, vanadium (III) chloride (VCL3), sodium hydroxide (NaOH), and free Hb—were American Chemical Society (ACS) grade and purchased from Sigma–Aldrich (St Louis, Mo.). Glacial acetic acid (ACS grade) and 12 M hydrochloric acid (HCl, ACS grade) were obtained from VWR International (Fayetteville, Ar.). Thereafter, 1-mM stock solutions of $NaNO_2$ and $NaNO_3$ were prepared in de-ionized (DI) water and 4 mM PBS were prepared by dissolving one 10X PBS packet in 1000 ml DI water with 2 g of glucose added to it. Saturated VCl3 solution was prepared by dissolving 0.8 g of VCl3 in 100 ml of 1 M HCl and DI water and a 0.2 M solution of NaI was prepared by dissolving 0.29978 g of NaI in de-ionized water.

Design of an in vitro experimental system for RBC-NO interaction studies.

In this study, a continuously stirred novel bioreactor device was designed that replicated in vivo conditions of NO generation and consumption in blood vessels. All the reactions were conducted using this experimental system (figures 1A and 1B). A 100-ml Pyrex bottle was used as the bioreactor with a controlled headspace and stirring device for the experimental solutions. The headspace cover included two openings (to serve as inlet and outlet for gaseous mixture) and a third sample collection port. These openings consisted of Swage-lock fittings glued into holes drilled in the bottle cover. Controlled gas-flow meters (Porter Instrument Co. Hatfield, Pa.) were used to balance the gas mixture and obtain a specified NO concentration from gas cylinders entering the bioreactor, where NO reacted with 10 ml of treatment solution. Flow rate of gas mixture into the headspace consisted of 100 ml/min of 100% ultra-high pure nitrogen and desired ml/min of 10% NO/N_2. The gas mixture was allowed to flow across stainless steel tubing to the inlet of the bioreactor in the chemical safety hood. The solution was continuously stirred with a magnetic stirrer at a constant speed of 2 rpm. To characterize the bioreactor in terms of NO delivery, we treated 10 ml of DI water at two different NO fluxes, and 100 µl samples were collected at 1, 2, 3, 4, 5, and 10 min. Nitrite concentration was measured in collected samples using chemiluminescence method. Next we conducted a variable-time experiment with 5% oxygenated RBC solution and measured both the nitrite and total NO_x concentrations. The ability of the device to study NO-RBC interactions was validated by treating oxygenated RBC solution at 5% and 45% hematocrit and measuring the respective reaction products.

Preparation and treatment of 5% and 45% RBC solutions.

Approximately 100 ml of pig blood were collected from the Savoy Experimental Farm, University of Arkansas, in 10-ml heparinized tubes (Vacutainer). Blood was centrifuged at RCF= 800g, RCM= 2713, Time= 10 min and Temperature= 4°C. Plasma was pipetted out and RBCs were washed thrice with 2-3 mL refrigerated PBS solution

(4 mM PBS + 2 g glucose). After separation of RBCs, the RBC/PBS solution was stored at 4°C to prepare the hematocrit solutions. Thereafter, 1 ml of RBC, obtained by centrifugation of pig blood, was mixed with 19 ml of prepared PBS solution to prepare 20 ml of 5% RBC solution, and 9 ml of RBC were mixed with 11 ml of PBS solution to obtain 20 ml of 45% RBC solution. For both the variable-time and variable-hematocrit experiments, 10 ml of the prepared RBC solution were treated for 10 minutes in the bioreactor under oxygenated conditions. Gas flow rates were adjusted at 100 ml/min of 100% N_2 and 0.7 ml/min of 10% NO/N_2 mixture, and the solution was constantly stirred.

Collection of samples.

For variable-time experiments, about 0.3 ml of blood samples were collected with a 1-ml syringe into eppendorf tubes at regular intervals of 2 min from the instant the NO/N_2 treatment (0-min blank sample) started till the end of the treatment (10-min sample). For variable-hematocrit experiments, only the blank blood sample (0 min) and the end sample (10 min) were collected. Then 1 μM, 5 μM, 10 μM, 50 μM, and 100 μM concentrations of $NaNO_2$ and $NaNO_3$ standard samples were prepared from the respective 1-mM stock solutions. The $NaNO_2$ and $NaNO_3$ standard curves were used to quantify the products NO_2^- and total NO_x, respectively.

Chemiluminescence method for measurement of reaction products.

The Model 280i Nitric Oxide Analyzer (NOA) from Sievers Instruments (Boulder, Colo.), a high-sensitivity detector with a detection limit of 1 picomole, was used for measuring NO based on a gas-phase chemiluminescent reaction between NO and ozone. The purge-vessel setup was used to detect NO_2^- and a gas-bubbler NaOH trap was added to the setup to measure total NO_x. Argon that bubbled through the purge vessel carried any released NO to the NO detector. NO reacts with dissolved oxygen to form NO_2^-. To measure NO_2^-, the purge vessel was filled with a reducing agent (l% wt/vol of NaI or KI in glacial acetic acid) to convert NO_2^- to NO, which was then carried to the NOA for detection. The reaction converting NO_2^- to NO,

$$I^- + NO_2^- + 2H^+ \rightarrow NO + l/2I_2 + H_2O \quad \text{(Equation 1)}$$

For most applications, ~8 ml (2 ml NaI and 6 ml acetic acid) of the reducing agent were prepared in the purge vessel, as this volume was sufficient for measurement of 20-50 samples, depending on the volume of the sample injected. The reagent was changed when the solution turned yellow due to formation of I_3^-, indicating depletion of the reducing agent.

NO reacts with oxyHb to form NO_3^-. To measure total NO_x, about 5 ml of vanadium (III) chloride in hydrochloric acid were used as the reducing agent to convert NO_3^- to NO,

$$2NO_3^- + 3V^+3 + 2H_2O \rightarrow 2NO + 3VO_2^+ + 4H^+ \quad \text{(Equation 2)}$$

To avoid foaming of the blood samples, 100 μl of antifoaming agent were injected into the reducing agent. To achieve high conversion efficiency, the reduction was performed at ~90°C.

For volumes corresponding to 100 μl of standard samples, 10 μl of blood samples for NO_2^- and 5 μl of blood samples for total NO_x were injected through a septum into the purge vessel to obtain concentration-dependent chemiluminescent signals in millivolts (mV). A calibration curve was prepared by plotting concentrations of standard solutions versus peak mV signals.

Statistics.

The DI-water experiments were conducted twice, changing the NO delivery rate. The experiments for 5% and 45% hematocrit were repeated three times under oxygenated conditions. The results presented were in the form of mean ± SE.

RESULTS AND DISCUSSION

Experimental system design.

The experimental system developed in the study was simple: to fabricate and reliably replicate the in vivo conditions of NO generation and consumption in blood vessels where flowing blood receives a constant NO flux from the endothelium, which forms the first layer of the vascular wall. The solution could be treated with a desired NO/N_2 flux for the complete duration of the experiment using the digitally controlled mass-flow meter and the setup could be modified to vary the NO+N_2 delivery rate into the solution in the bioreactor. The sample collection port on top facilitated a convenient withdrawal of sample from the solution without significant disturbance to the experimental conditions. The gas outlet port prevented buildup of gases in the bioreactor. Continuous stirring of the solution minimized effects of any effective diffusional barrier due to the unstirred layer surrounding the RBC, which is responsible for preserving NO bioactivity (Liu et al., 2002).

So far the majority of studies have been performed using NO-delivery systems like direct injection of saturated solutions of NO (Han et al., 2002; Joshi et al., 2002; Han et al., 2004) and NO donors (Vaughn et al., 2000). Disadvantages of using these direct-delivery systems are that they can cause significant loss of NO from the exper-

imental system (Kavdia and Lewis, 2003) and create high, localized concentrations of NO that react instantaneously before reaching uniform concentration in the system. They also contain nitrite as an impurity, which can affect the interpretation of RBC-NO interaction products (Nagababu et al., 2003). This in vitro, controlled, gaseous NO-delivery system helped to overcome technical difficulties involved in in vivo determination of local availability of the gas, maintained a homogeneous concentration of NO in the reaction environment, and also eliminated the possibility of product contamination. The system thus allowed RBC-NO reactions to take place as a steady state was maintained on both the liquid side and gaseous side, which more accurately simulated the physiological conditions in microcirculation.

Nitrite and total NO_x standard curves to quantify the treated samples.

Standard samples of NO_2^- and NO_3^- were injected into the NO analyzer to generate standard curves for both nitrite and total NO_x concentrations, respectively. Fig. 2A shows the chemiluminescent signal (mV) recorded by the NO analyzer after injecting standard nitrite samples in concentrations of 1, 5, 10, and 50 μM. Fig. 2B shows the peak mV value for each concentration of standard nitrite sample. Similarly, figures 3A and 3B show the chemiluminescent signal and peak mV signal, respectively, for total NO_x concentrations of 1, 5, 10, 50, and 100 μM.

The peak mV signals showed a linearly increasing trend with increases in nitrite and total NO_x concentrations. The standard curve data established a linear relationship between the standard concentration and peak mV signal. Thereafter, before injecting treated samples, a 1-μM standard sample was injected every time the reducing agent in the purge vessel was changed. The concentration of the treated sample was calculated using the formula,

$$\frac{[\text{Sample peak signal (mV)} - \text{Sample base signal (mV)}]}{[\text{Standard peak signal (mV)} - \text{Standard base signal (mV)}]} \times \text{Dilution factor}$$

(Equation 3)

where the base signal was the lowest value recorded before injecting the sample or standard and the peak value was the highest mV signal obtained after injecting the sample or standard.

DI water experiments to characterize the NO-delivery rate of the experimental system.

To characterize the experimental system, we conducted experiments by treating 10 ml DI water in the bioreactor and studied the effect of changing the gaseous partial pressure in headspace on the total NO-delivery rate (moles/min). Fig. 4A shows the NOA signal recorded for the nitrite analysis of DI water samples collected at reaction times 0, 2, 4, 6, 8, and 10 min, when treated with a flow rate of 0.7 ml of 10% NO/N_2 + 100 ml of 100% N_2. Fig. 4B shows the nitrite concentration profile for DI water samples collected at regular reaction-time intervals. Similarly, figures 5A and 5B show the NOA signal and nitrite-concentration profile for DI water samples collected at reaction times 0, 1, 2, 3, 4, 5, and 10 min, conducted at flow rates of 1.5 ml of 10% NO/N_2 + 100 ml of 100% N_2.

From the best-fit trend-line (figures 4B and 5B), we noted a linear increase in nitrite concentration with each successive time sample injected from 0 min to 10 min for both cases. Slope obtained from the best-fit linear trend-line gave the delivery rate of NO in units of μM/min. NO delivery rate was converted to moles/min using the relationship,

$$\textit{moles} = \textit{concentration} \times \textit{volume} \quad \text{(Equation 4)}$$

where the volume was 10 ml. NO-delivery rates calculated using Equation 4 were 3.853 nmoles/min for a flow rate of 0.7 ml of 10% NO/N_2 + 100 ml of 100% N_2, and 22.22 nmoles/min for a flow rate of 1.5 ml of 10 % NO/N_2 + 100 ml of 100% N_2.

The results indicated that the NO-delivery rate increased about 5.77 times as the 10% $NO+N_2$ flow rate increased from 0.7 ml/min to 1.5 ml/min. Nitrite concentration was time-dependent. With an increase in time during which the DI water solution was allowed to react with NO/N_2 gaseous mixture, there was a linear increase in the nitrite concentration in the solution. The NO reacts with dissolved O_2 to form nitrite, with a reaction rate $KC_{NO}^2C_{O2}$, where $K = 9.8 \times 10^6\ m^{-2}s^{-1}$. This implied that the experimental system was able to maintain a constant NO-delivery rate in the solution during the entire duration of the treatment and NO was allowed to mix homogenously throughout the stirred solution. Thus our system has an advantage over systems in which addition of NO bolus causes rapid initial reaction before NO is homogenously mixed throughout the solution (Joshi et al., 2002). The results proved that this in vitro system can be used to treat RBC solution with a constant NO flux, thereby more accurately replicating in vivo conditions of RBC-NO interactions. Thus using these preliminary data of nitrite formation with DI water, we were able to characterize the experimental system.

Nitrite and total NO_x levels in 5% oxyRBC solution.

Next, we treated 10 ml of 5% oxy RBC solution with 0.7 ml of 10% NO/N_2 + 100 ml of 100% N_2 for 10 min, regularly collecting samples at reaction times of 0, 2, 4, 6, 8, and 10 min. Figures 6A and 6B show the nitrite- and total NO_x-concentration profiles, respectively, for these

samples. The results showed that as treatment time increased from 2 min to 10 min, concentrations increased from 1.23 μM to 2.79 μM for nitrite and 10.3 μM to 25.1 μM for total NO_x. There was a linear increase in nitrite and total NO_x concentrations as reaction time between 5% oxy RBC and NO increased. Figure 6C shows percent ratio of nitrite to total NO_x in the 5% oxy RBC samples at different time points. The ratio remained almost constant at 11.87 ± 0.876% for 2-10 min.

Results indicate that our system maintained a steady-state at both gaseous and liquid levels in the bioreactor. Oxygenated RBCs rapidly scavenged NO at an uptake rate of 21.9 nmoles/min calculated from the total NO_x linear-concentration profile. Also nitrite was formed as a relatively minor portion of the total NO_x species, suggesting that the major part of the reaction products formed were either in the stable nitrate form or other intermediate species like S-nitrosithiols. Although nitrite is the main product of NO oxidation in blood plasma (equation 5), upon interaction with oxygenated heme domains in RBC, however, NO is rapidly oxidized to form nitrate and metHb (equation 6a). Our experiments suggest that the oxidation product nitrate is formed at a higher concentration than the conservation product nitrite. Similar observations were reported by Liao and colleagues, who conducted experiments by generating NO homogenously using NO donors (Joshi et al., 2002). Thus nitrite and other NO-species like SNOHb, a product of s-nitrosation (Equation 6b), and HbFe(II)NO, a product of reductive nitrosylation (equations 6c and 6d), may be formed that contribute to the conservation of NO bioavailability in the microcirculation (McMahon et al., 2002), but the consumption reaction dominates the RBC-NO interaction in physiological conditions.

Oxidation of NO,

$2NO + {}_{1/2}O_2 + H_2O \rightarrow 2NO_2^- + 2H^+$ (Equation 5)

Reaction of NO with oxyRBC,

NO+oxyHb [HbFe(II)O_2] → metHb [HbFe(III)] + nitrate [NO_3^-] (Equation 6a)

S-nitrosation of metHb,
metHb [HbFe(III)] + NO → SNOHb (Equation 6b)

Reductive nitrosylation of metHb,

metHb [HbFe(III)] + NO → NO^+ + HbFe(II)
(Equation 6c)
HbFe(II) + NO → HbFe(II)NO (Equation 6d)

Effect of variable hematocrit on nitrite and total NOx levels in oxygenated RBC solution.

Products of the NO reaction with the RBC-encapsulated Hb depend on the concentration of the hematocrit and to study this effect, we conducted experiments by varying hematocrit from both 5% to 45% in oxygenated conditions. Three separate sets of experiments were conducted and mean values were plotted with standard error bars. Figures 7A and 7B show the comparison of nitrite and total NO_x concentrations, respectively, for samples collected at 10 min for each hematocrit percentage. The mean nitrite concentrations increased from 3.73 ± 1.17 μM for 5% hematocrit to 5.61 ± 3.73 μM for 45% hematocrit. Total NO_x concentrations also increased from 34.4 ± 10.82 μM for 5% to 40.2 ± 7.68 μM for 45% hematocrit. Mean nitrite concentration increased 1.5 times and total NO_x concentration increased 1.17 times as the hematocrit was increased from 5% to near physiological range of 45%. Figure 7C shows the comparison of nitrite to total NO_x-percent ratio for the two oxy RBC solutions. The ratio also increased from 10.84 ± 4.28% for 5% hematocrit to 13.96 ± 10.93% for 45% hematocrit solution.

As NO reacts homogenously with increased concentration of RBC in the solution, there is an increased scavenging of NO by RBC-encapsulated Hb, thereby increasing the concentration of total NO_x (Azarov et al., 2005). Nitrite is formed as a product of oxygenation reaction of NO with O_2 in solution (equation 5) before NO can be rapidly scavenged by RBC-encapsulated Hb. Availability of higher levels of O_2 in oxygenated condition and more O_2-bound heme Fe (II) sites to react with NO at higher hematocrit results in an increase of the nitrite concentration in oxygenated solution. Nitrite was formed in the range of 11 to 13% of the total NO_x species. Formation of the conservation product, nitrite, as a minor species indicates that NO and oxyRBC interaction is dominated by the consumption reaction to form metHb and nitrate (equation 6a). The dominance of the consumption pathway was observed by Gladwin and colleagues, and also in experiments conducted by Liao and colleagues using NO donors (Gladwin et al., 2000; Han et al., 2004). Liao and colleagues further reported that in experiments conducted using saturated NO bolus, intermediate species like SNOHb and HbFe(II)NO were formed as minor portions of the total NO_x species. But research groups have reported different values for concentration of these minor species (McMahon et al., 2002), and have indicated that since these conservation species form a very small portion of the total NO_x species, their physiological significance in preserving NO remains unclear. Hence, using the experi-

mental system designed in this study, we found that NO-RBC interactions were dominated by the consumption reaction, forming nitrate and metHb [HbFe(III)].

Conclusions

Our results help to explain the mechanism of physiological regulation of NO bioactivity by alteration of RBC concentration and NO delivery rate, and thereby significantly increase our understanding of the interactions of NO with RBCs in the vasculature. The experimental setup allowed maintaining a steady state in the bioreactor as a constant NO flux reacted with the RBC solutions, thus effectively replicating the in-vivo conditions in microcirculation. The setup also could be easily modified to control the NO flux delivered into the solution. Results showed that for oxygenated solution, the nitrite and total NO_x concentrations increased as the hematocrit increased from 5% to 45%. This deeper understanding of the multiple facets of RBC and NO biology will provide important insights into mechanisms of vascular homeostasis and will offer novel therapeutic strategies for treatment of vascular-related pathologies

ACKNOWLEDGMENTS

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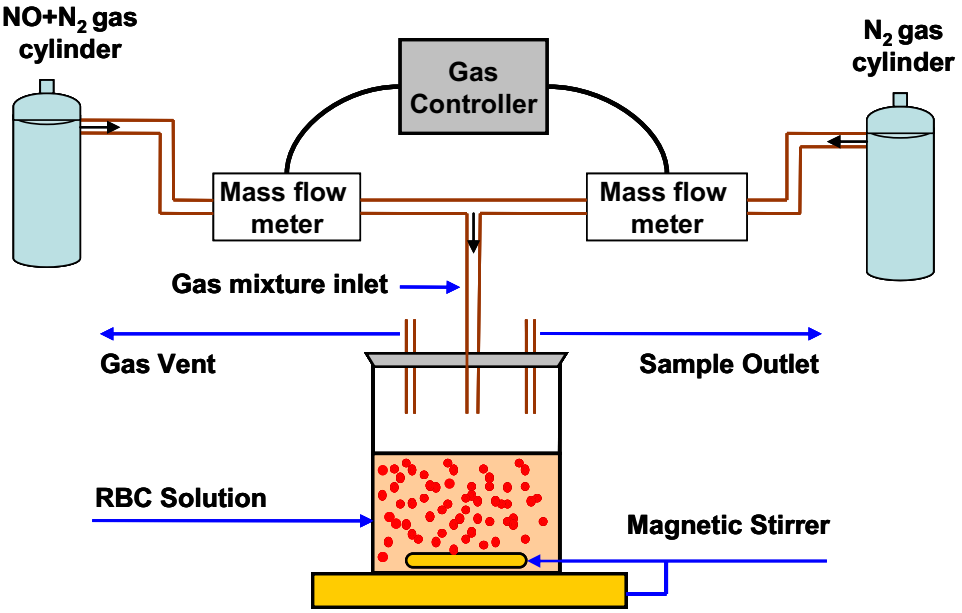
A.



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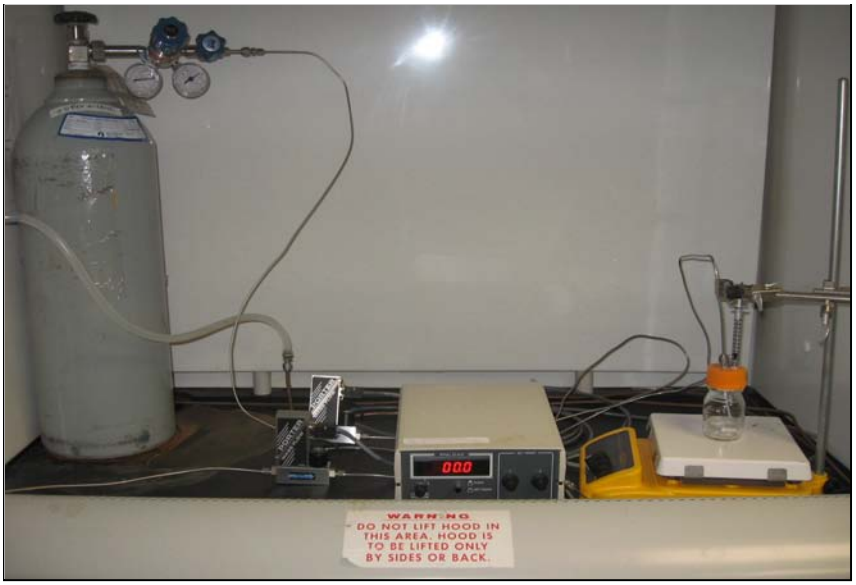


Fig. 1. A. The schematic of the experimental system.
B. The experimental system setup in the laboratory.

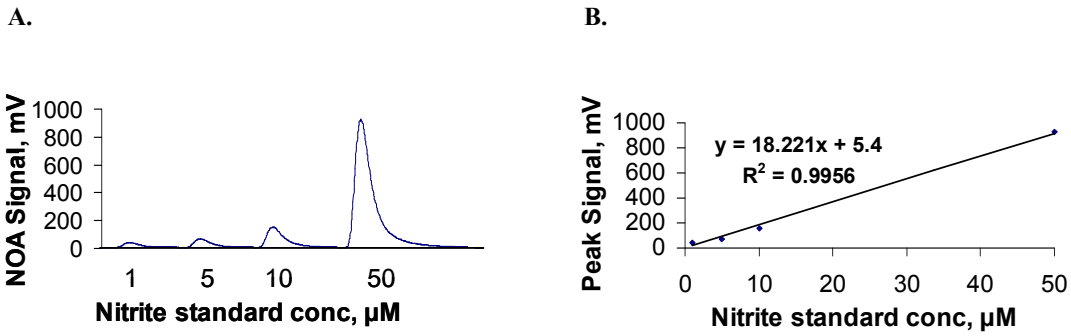


Figure 2: **A. Chemiluminescence signal for nitrite standard samples. B. Calibration curve for the nitrite standards.**

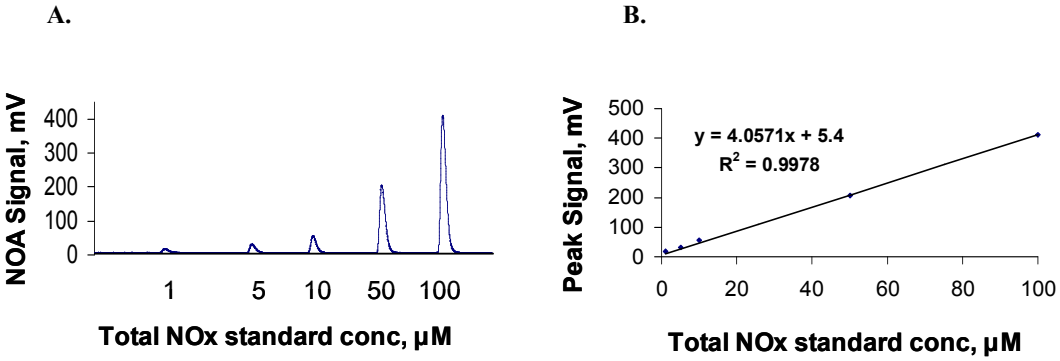


Figure 3: **A. Chemiluminescence signal (mV) for total NOx standard samples. B. Calibration curve for the total NOx standards.**

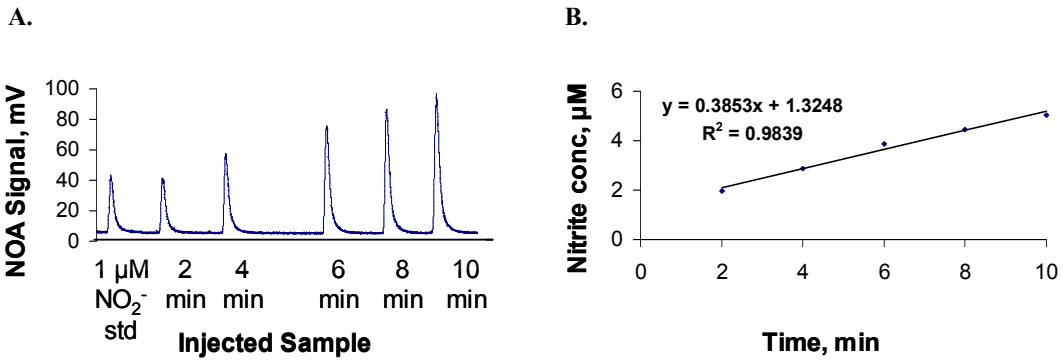


Figure 4: **The figures show results of DI water experiment;**
Flow rate: 0.7 ml of 10% NO/ N_2 + 100 ml of 100 % N_2;
A. Chemiluminescence signal (mV) from NOA;
B. Nitrite concentration profile for DI water samples taken at regular time (min) intervals.

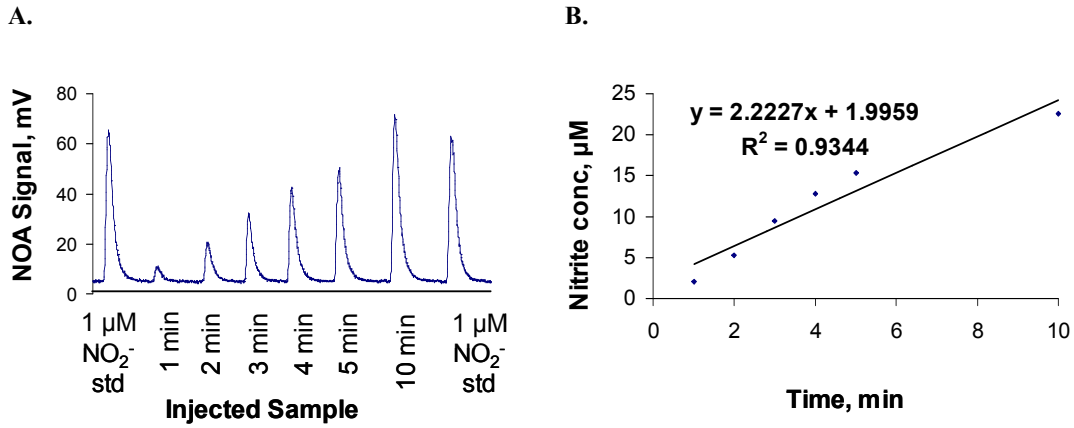
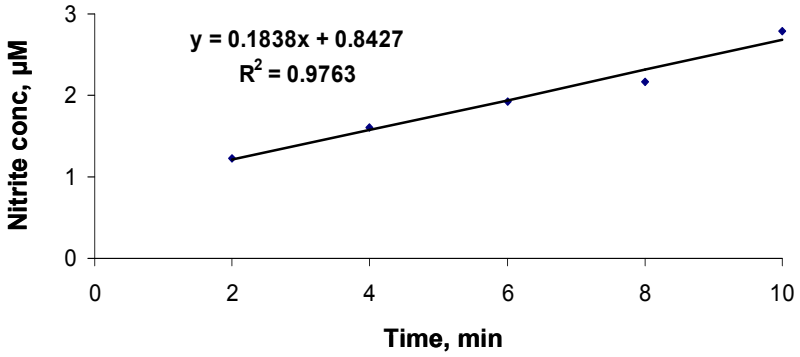


Figure 5: **The figures show results of DI water experiment;**
Flow rate: 1.5 ml of 10% NO/ N_2 + 100 ml of 100 % N_2;
A. Chemiluminescence signal (mV) from NOA;
B. Nitrite concentration profile for DI water samples taken at regular time (min) intervals.

A.



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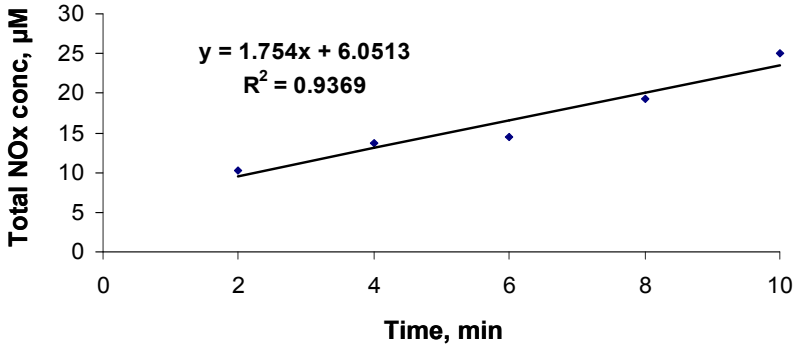

C.

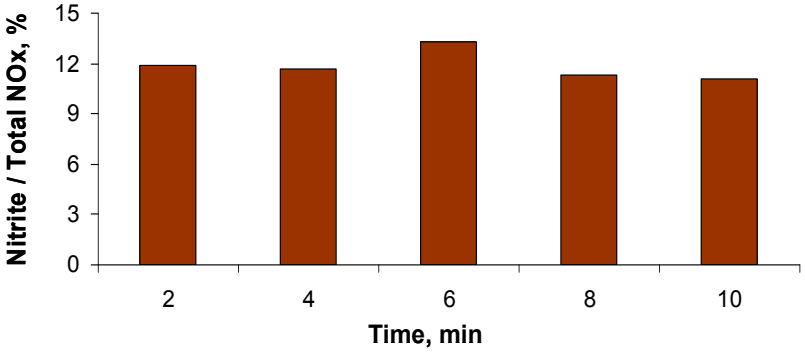

Figure 6: The figures show results of 5% oxy RBC variable time experiment.
Flow rate: 0.7 ml of 10% NO/ N_2 + 100 ml of 100 % N_2; Total Time: 10 min
A. Nitrite concentration profile;
B. Total NOx concentration profile;
C. Nitrite to total NOx percent ratio for samples taken at regular time (min) intervals.

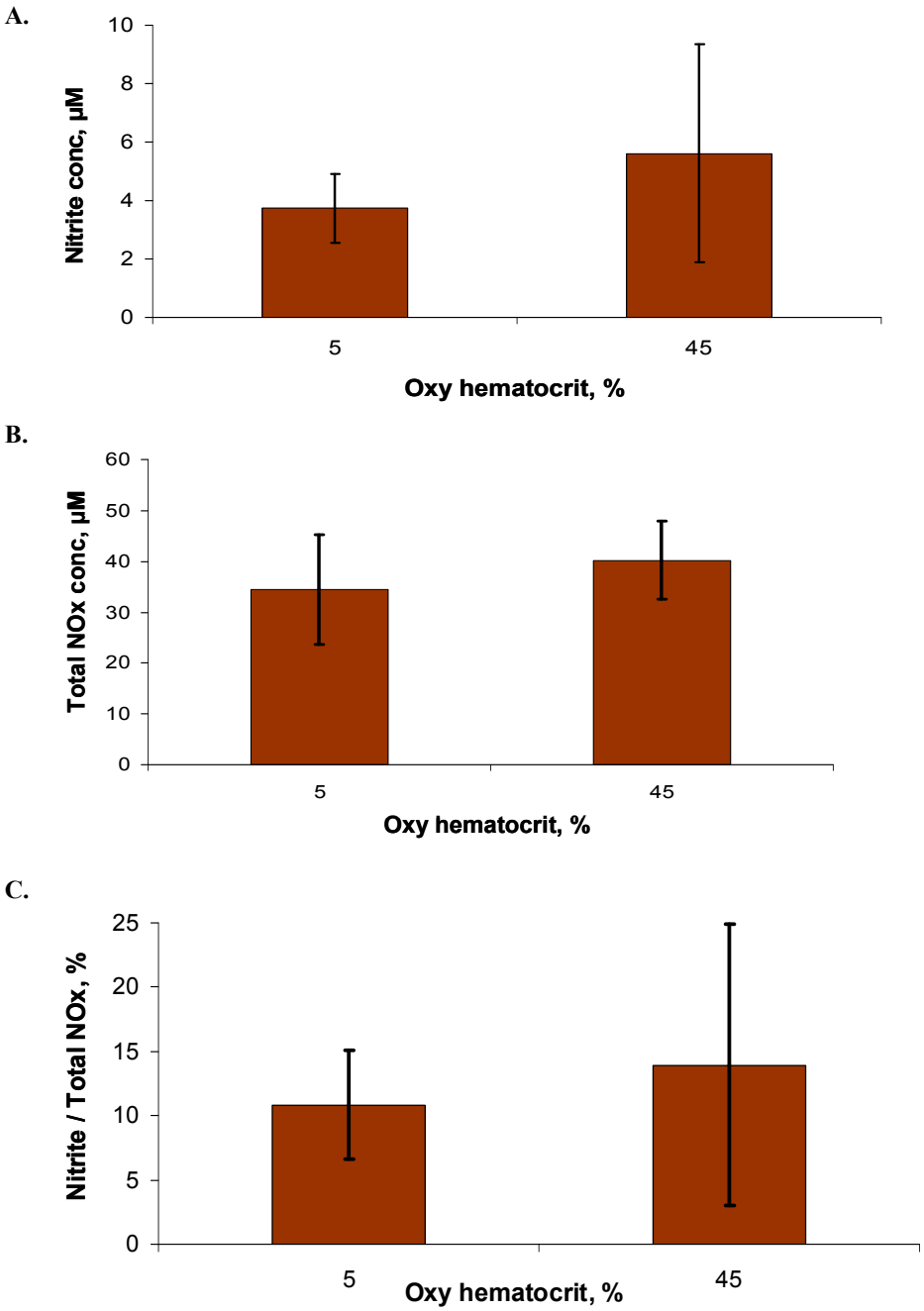

Figure 7: **The figures show results of variable Ht experiments in oxygenated conditions**
Flow rate: 0.7 ml of 10% NO/ N_2 + 100 ml of 100 % N_2; Total Time: 10 min
A. Nitrite concentration in 5 and 45 % Ht oxy solution
B. Total NOx concentration in 5 and 45% Ht oxy solution
C. Nitrite to total NOx percent ratio in 5 and 45% Ht oxy solution

Arkansas producers' attitudes toward the 2002 Farm Bill and preferences for the 2007 Farm Bill

Misti Clark and Eric Wailes* †

ABSTRACT

The Federal Security and Rural Investment Act of 2002, otherwise known at the 2002 Farm Bill, contains current legislation regarding federal public policies and programs for U.S. food and agriculture. This legislation will expire in 2007 and thus new legislation will be developed. It is important to have farm producers' input for developing this legislation because the policies and programs influence their business practices and livelihoods. The purpose of this study was to determine Arkansas producers' attitudes toward current and future farm legislation based on an analysis of a survey administered to Arkansas farm producers in summer 2006. The main finding of this research is that Arkansas producers would like to create more incentives for biofuel research. They also indicate through survey preferences that risk management policies such as insurance, disaster assistance, and labeling of foods should be addressed more thoroughly with more funding allocated to these areas. Arkansas producers are not in favor of eliminating current commodity payments although there was a significant difference of opinion in this area between those who produce program crops and those who do not. These study results provide an important assessment of producer preferences for future farm legislation.

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INTRODUCTION

The *Federal Security and Rural Investment Act of 2002*, otherwise known as the 2002 Farm Bill, contains the current legislation regarding federal public policies and programs for United States food and agriculture. This legislation will expire in 2007 and thus new legislation will be written to replace this legislative act. It is important to have producers' input to develop this legislation because the policies and programs influence their business practices and livelihoods. Without knowledge of farmers' attitudes towards this legislation, it will be difficult to develop policies that strengthen and stabilize the agricultural economy for Arkansas. Arkansas agriculture accounts for 20 percent of the total value of the Arkansas economy (Popp, Kemper and Miller).

In 2002, a survey was developed to investigate producers' attitudes toward the existing farm legislation and the development of the 2002 Farm Bill. This study was based on responses to the survey questionnaire sent to farmers in participating states. The survey responses were analyzed and reported at the national, regional and state levels to indicate producers' preferences for the 2002 legislation (Lubben, et al., 2001). Arkansas did not participate in the 2002 study. However, a similar survey was implemented in most states, including Arkansas, in 2006 to identify preferences for the 2007 Farm Bill. The Arkansas field office of the National Agricultural Statistical Service (NASS) of the United States Department of Agriculture (USDA) implemented the survey in Arkansas (Cochran). As a result of delay in implementation, results for Arkansas were not included in the National Report (Lubben et al 2006).

The objectives of this paper are to analyze attitudes of Arkansas farm producers about the 2002 Farm Bill and their preferences for new 2007 legislation. The study determines key value differences among Arkansas producers as well as develops a comparison for those producers in the United States and other southern states based on the report given by Lubben et al. The null hypothesis is that there is no difference among producers in their attitudes and preferences for the Farm Bill. An alternative hypothesis is that there are differences among producers that can be explained normatively by differences in past participation in farm programs.

MATERIALS AND METHODS

NASS distributed the survey in Arkansas to 2,400 operations in three different strata. These strata included (1) producers making less than $100,000 in farm sales, (2) producers making between $100,000 and $249,999 in farm sales, and (3) producers making more than $250,000 in farm sales. Thirty percent of the samples were drawn from the first stratum and 30% were

Misti Clark

MEET THE STUDENT-AUTHOR

Born in Madison County, Ark., but raised in Washington County, Ark., I always claim to have two home towns. I graduated from Prairie Grove High School in 2004 and enrolled in the University of Arkansas the same year in the Department of Agricultural Economics and Agribusiness. I will graduate in May 2008 with a B.S. in agribusiness management and marketing as well as with minors in Spanish and global agriculture. I have served as secretary of the professional agricultural sorority, Sigma Alpha, and I am currently the president of this organization. I am also involved in the Agribusiness Club and was recently elected as the vice-president. I was awarded a State Undergraduate Research Fellowship under my mentor, Dr. Eric Wailes. This research has helped me to further develop my professional and scholarly goals. My areas of interest are rural development and policy, and I would like to get more experience in these areas through graduate school and a PhD program. After these programs, I would like to become a professor to continue doing helpful research in these important areas.

drawn from the second stratum, with the remaining 40% drawn from the third stratum. Each response was assigned a weight in order to correct for under-sampling of the Arkansas farm population in stratum one and over-sampling in strata two and three. A second mailing of the questionnaire was sent to all non-respondents to the first distribution. The responses were sent directly to the Department of Agricultural Economics and Agribusiness at the University of Arkansas.

After the surveys were received from respondents, responses were entered into a Microsoft Access database that was then transferred to JMP® and SAS, statistical software packages. Comparisons were made between producers who produced program crops and those who did not. Program crops are crops eligible for government price and income support payments authorized by the Farm Bill. In Arkansas, key program crops are cotton, corn, rice, soybeans, sorghum, and wheat. Also, the researcher compared responses considering whether the respondent had received government program funding previously from commodity payments and environmental/conservation programs that might have affected the responses on questions about these policies. No additional comparisons by demographic characteristics were made given the homogeneity of the respondents. An overwhelming majority of respondents were within the same age range, race, and educational level.

RESULTS AND DISCUSSION

Farm Programs and Budget Priorities

Producers were first asked to rank goals of the Farm Bill with 5 being most important and 1 being least important. For simplicity in presentation, responses to each question were combined in two groups, important or most important compared to all other responses. The most important goals indicated by Arkansas producers were those of assuring a safe, secure, abundant, and affordable food supply, and reducing the nation's dependency on non-renewable sources of energy. In both areas, about 88% of the respondents agreed that it is important/most important to prioritize these as goals in the upcoming Farm Bill. In general, however, respondents agree that all of the goals listed are important for the upcoming legislation. The area with the least support was that of protecting the nation's land, water, and environmental policies, with 68% still agreeing that this should be a major goal for the upcoming Farm Bill (Table 1). One interpretation is that this is an issue that respondents might think does not need as much discussion in policy debate because this issue has been adequately dealt with through past legislation. Other issues that drew strong support (72-83% of respondents indicating important or most important goal) for upcoming legislation were the areas of enhancing farm income, reducing price/income risk, increasing global competitiveness, creating opportunities for small farms, and enhancing rural economies.

Arkansas producer responses are similar to those in the southern region as well as those throughout the nation. Producers in Arkansas and the rest of the country indicated very strong support for reducing the nation's dependency on non-renewable sources of energy. Less important for agricultural policy for producers in Arkansas and in the rest of the country is the goal of reducing price/income risk and the goal of protecting land, water, and environment.

Among Arkansas producers, program crop producers and non-program producers were significantly different on two goals. Producers of program crops more strongly supported the goal of reducing price and income risks than did non-program producers. On the other hand, non-program producers more strongly supported the goal of providing opportunities for small farms than did program crop producers.

The next set of questions asked producers to indicate the importance of funding for specific government programs. Producers were asked to rank the importance of programs currently funded. A program that Arkansas producers would like to continue funding is that of disaster assistance programs with 75% of Arkansas producers indicating that this is important/most important. Producers also favor continued funding for agricultural credit programs/FSA loans (57%) and risk management programs for crops and livestock insurance programs (56%). There were significant differences between program crop producers and non-program crop producers in Arkansas pertaining to the importance of maintaining funding for several current farm payment programs including direct payments, counter-cyclical payments (CCPs), loan deficiency payments (LDPs), and land conservation (Fig. 1). The program crop producers rated commodity payment programs much higher than did the livestock producers. Program crop producers agree that funding should be maintained for fixed (direct payments) (87%), CCPs, commodity loans, and LDPs (88%). They were also more in favor of keeping land retirement conservation programs such as the Conservation Reserve Program (CRP) and the Wetland Reserve Program (WRP) (50%) than were non-program crop producers (37%). Of the program crop producer respondents, 90% received government payments within the last year. Only 6% of non-program crop respondents received benefits from the land conservation programs last year. In general, non-program crop producers in Arkansas were in favor of changing the monetary dis-

tribution in the upcoming 2007 Farm Bill from program payments to other areas such as conservation and risk management.

Producers were asked to rank the importance of providing new or reallocated funds for a set of alternative programs. The program for new or reallocated funding most favored by Arkansas producers was that of providing incentives for bioenergy production (75%). The other two important issues for respondents were those of food safety programs and assistance (69%) and biosecurity incentives and assistance (59%). Respondents either did not support or were neutral about new or reallocated funds toward payments tied to farm income levels, payments for currently non-funding commodities, and traceability and certification programs.

Nearly a quarter of producers in the Arkansas sample have produced food and feed grains, soybeans, or both within the past year. These farmers could qualify for the aforementioned bioenergy incentives because they already produce the materials that are being used commercially to produce biofuels. The issue of alternative fuels was also of great concern to producers because of the rising cost of fuel for producers. The percentage of costs on a farm for fuel has continued to rise over recent years. As a rising concern for producers, Arkansas would like to see some relief from high fuel prices and would also like to benefit directly from the development of biofuels made from grains and oilseeds.

Commodity Programs and Risk Management Policy

Current commodity programs, particularly trade-distorting subsidies like the LDPs (loan deficiency payments), are a contentious issue in the current Doha Development Round negotiations in the World Trade Organization (WTO). The G20 and G33 groups are pushing for the United States to reduce these subsidies (WTO, 2006). This, however, is not something that Arkansas producers support. Arkansas producer-respondents generally agreed that new policies should not reduce or eliminate commodity payments, including LDPs, CCPs (counter cyclical payments), and direct (decoupled) payments. However, there were significant differences between program crop producers and non-program crop producers (Table 2). Those who did not produce programs crops were less supportive of maintaining the trade-distorting program subsidies than were the program crop producers. Program crop producers strongly disagreed (88%) with phasing out farm commodity payments over the length of the 2007 Farm Bill. Non-program crop producers were more supportive in the areas of targeting commodity payments to small farmers (68%), tying commodity payment limits to a single individual (65%) and eliminating the unlimited use of generic certificates and forfeiture gains that are used to increase program crop payments (53%). Program crop producers were more in favor of maintaining funding for the milk subsidy programs than were the non-program crop producers. A slim majority of producers (51%) seemed to agree that new legislation should also reauthorize both the current dairy price-support program and the MILC (milk income loss contract) program.

Producers were also asked in this section if they would be in favor of a buy-out program that would offer producers a lump-sum payment or series of payments in exchange for eliminating all future commodity program payments (Fig. 2). The producers did not support the option of buying-out current commodity payments. The only option in the survey that produced a positive majority was that of accepting a lump sum worth 25 years of current payment in today's dollars (63%). Producers did not favor eliminating these program crop subsidies. It is important to note the large number of "don't know/no opinion" responses to the buy-out option questions. In Arkansas, there were no less than 42% missing values on each of the buy-out questions. Nearly 40% of the U.S. producers responded "no opinion/don't know" to every buy-out option. If new farm legislation were to include a buy-out program, producers would have to be more informed about the option before many would likely support this policy approach.

Conservation and Environmental Policy

The third section of the survey examined farmers' opinions regarding conservation and environmental policies. The first question asked producers whether federal technical and financial assistance should be offered to producers in the areas of water quality, wildlife, biodiversity, and other areas to assist with meeting environmental and conservation goals. In general, Arkansas producers favored assistance for most programs listed in the survey with water quality and soil erosion being the most favored areas (71% and 63%, respectively). In addition, producers indicated a high response of "don't know" for the questions about carbon sequestration and maintenance of biodiversity. Arkansas producers were concerned about environmental issues and would like to do something to help, however they will need assistance, including monetary and technical support. The U.S. survey responses were similar with technical/financial assistance being the most popular choice.

Another question in the environmental program area was whether funding for conservation programs should be given to the states in the form of block grants to give individual states more authority over implementation of conservation programs. Sixty percent of the respondents agreed that the funds should be reallocated to the states to give the states more control of the implementation of

funds for these conservation programs. The producers in the national and the southern regional samples were almost identical in their responses on this topic. One may conclude that a large number of producers would prefer that individual states be given more discretion to manage state-level environmental programs.

Preferences in the area of the Conservation Reserve Program (CRP) and the Conservation Security Program (CSP) were examined to determine what Arkansas producers would prefer regarding these programs. The highest support for CRP (39%) was to keep current rules and allow current contracts to expire on schedule and compete for re-enrollment against other land being offered for re-enrollment (Fig. 3). Another popular response (25%) was to completely eliminate the CRP as current contracts expire. This option was only the third highest ranked option for the southern states and U.S. The reason why Arkansas producers would be more likely to eliminate this program is because only about 10% of Arkansas respondents received benefits from the CRP last year. This is a lower priority than other programs that are more highly practiced in Arkansas. The respondents seemed more in favor of continuing with the current policies of the CSP on a watershed basis as funding allows (Fig. 4).

Trade Policy

Trade policy is an issue that affects many agricultural producers, particularly the producers in Arkansas as they are the leading group of rice exporters in the U.S. Producer-respondents in Arkansas were very much in favor of including labor laws, environmental impacts, and food safety standards as part of international trade negotiations (74%), continuing to pursue free trade (60%), and eliminating unilateral sanctions of food trade (52%). They were generally not in favor of withdrawing from the WTO (73% indicated disagreement or neutrality) and they believe that if we were to withdraw, we would experience market access losses and agricultural export problems (55.5%). Arkansas producers disagreed or were neutral about complying with the WTO ruling on cotton and eliminating Step 2 cotton payments (63% indicated disagreement or neutrality) as well as the issue of whether the U.S. should emphasize domestic economic- and social-policy goals rather than trade policies (63% indicated disagreement or neutrality).

Compared to the U.S. sample, the same issues received similar levels of support. The strongest support in trade policy for Arkansas, southern-state, and national producers was to include labor, environment, and food safety standards in international trade negotiations. The next highest support for all areas was that of continuing to pursue free trade with the realization that withdrawal from the WTO would result in market access problems for exports to other countries. The lowest ranking trade issue for all respondents in Arkansas, the southern states, and the nation is to comply with the recent WTO ruling on cotton. Overall, producers are interested in expanding trade, but are not necessarily as interested in being held accountable to the WTO rules included in trade agreements. The producers would also like to see some reforms in trade policies so that they include the areas of human welfare and food safety.

Food System and Regulatory Policy

The next area of questions pertained to topics that are of recent interest to the producers not only in Arkansas, but across the U.S., including questions about country-of-origin labeling (COOL) as well as animal ID, Bovine Spongiform Encephalopathy (mad cow disease) testing, and biotechnology labeling. Eighty-five percent of Arkansas respondents agreed that the government should implement mandatory COOL labeling on all food products. They also agreed (70%) that the government should increase efforts to improve traceability of food products from the consumer back to the producers. Issues of biotechnology seem to be an important issue also, with the majority favoring labeling of all biotechnology food, no matter the degree of genetic modification (58%). They were less concerned with government intervention in the area of BSE testing (59% responded *disagree/neutral*), and with government-implemented animal identification (53% responded *disagree/neutral*).

Related Policy Issues

The final section of the survey questioned opinions on issues of importance to Arkansas. Arkansas producers were asked about current agricultural issues such as credit extension and the allocation of research funds. Producers indicated that there are adequate supplies of funds from commercial lenders (63%) and that there is also adequate competition among agricultural credit suppliers (57%). However, they believe that the Farm Service Agency's (FSA) guaranteed loans to beginning farmers are too low (39%), and they think that the FSA direct loans are just right or too high (76%). They responded that the cap for the FSA direct loans is too low (43%). They also indicated that only those who bought at least the minimum amount of disaster insurance should be able to get the FSA emergency disaster loans (56%). Overall these data indicate that Arkansas producers are fairly happy with current credit availability and programs but think that more help should be given to beginning farmers and that lenders should lower the caps for direct loans.

In the area of research, Arkansas producers were interested in funds being put towards almost all areas of research. The most important research area for Arkansas respondents was biofuels and renewable energy (90%).

Research on water quality and food safety was the second and third highest ranking issue (83% and 82%, respectively). Areas receiving 60-79% support include the areas of production agriculture, food security, biotechnology, biosecurity, nutrition and obesity, air quality, and soil quality. The areas that were ranked as least important were private forestland management and community and economic development. Sixty percent and 52%, respectively, responded with disagreement or neutrality in funding in these areas.

Conclusions

In general, many producers in Arkansas believe that the priorities of the 2007 Farm Bill should focus on programs with particular emphasis on renewable sources of energy and assuring a safe, secure, abundant, and affordable food supply. Disaster assistance is also an important issue for Arkansas producers. This is an important issue because many of the respondents from Arkansas received more disaster assistance within the last year than respondents from any other state in the survey. One of the lesser concerns for Arkansas producer-respondents was reducing price and income risks. This could simply mean that renewable sources of energy take precedence over income risk right now, or it could mean that they believe current policies do a good job of minimizing these risks. Even though producers did not indicate that they valued the goal of income risk security, their responses in other areas show that this is not the case. Arkansas respondents indicated that they would not like to reduce or eliminate commodity program payments. The only way that they would be in favor of a buy-out program similar to the tobacco program would be if they were to be given a lump sum worth 25 years of current payment in today's dollars. In other words, these producers believe the government should focus on developing new technologies, while at the same time continue to help secure the future of agriculture by means of income supports.

Other programs including dairy programs, conservation programs, and trade agreements were supported by the respondents. Many environmental goals will require further assistance, especially in the areas of water quality and soil erosion. Farmers were in favor of keeping the current CRP and CSP programs and their rules. Arkansas producers were in favor of free trade, although they supported reform in the areas of labor laws, environmental impacts, and food safety in trade agreements. In the areas of regulatory policy, credit extension, and research, Arkansas producers were favorable toward new ideas that could make production safer as well as inform the public about the products that the public consumes. Country-of-origin labeling (COOL), animal identification, biotechnology labeling, and BSE testing are all new regulatory policies that Arkansas producers support. Producers felt it was important to have COOL labeling and animal identification. They were less in favor of BSE testing being done by the government, but think it needs to be done on the private level. Respondents agreed that credit extension in Arkansas could be better if the availability for new producers was increased. The allocation of research funds is consistent with other goals throughout the survey. Producers want to see more research in the areas of biofuels, food safety and security, and biotechnology.

ACKNOWLEDGMENTS

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Table 1. Importance of Farm Bill goals for producers in Arkansas, U.S., and southern states

	Arkansas Mean Response	National Mean Response	Southern Mean Response
Goals of the Farm Bill			
Enhance farm income	4.19	4.080	4.18
Reduce price/income risk	3.96	3.850	3.92
Increase global competitiveness	4.31	4.190	4.28
Opportunities for small farms	4.26	4.320	4.34
Protect land, water, and environment	3.87	3.980	4.07
Enhance rural economics	4.04	4.030	4.07
Assure safe, secure, affordable food supply	4.51	4.290	4.5
Reduce dependency on non-renewable energy	4.5	4.320	4.29
*Importance was rated from 1 (least important) to 5 (most important)			

Table 2. Preferences for new farm programs

	Arkansas Mean Response	National Mean Response	Southern Mean Response
New or Reallocated Funds			
Support payments tied to farm income levels	3.39	3.450	3.460
Payments for currently non-funded commodities	3.17	3.060	3.260
Incentives for farm savings accounts	3.46	3.390	3.580
Bioenergy production incentives	4.06	3.780	3.790
Biosecurity incentives and assistance	3.69	3.410	3.480
Food safety programs and assistance	3.9	3.710	3.880
Traceability and certification programs	3.22	3.280	3.360
*Importance was rated from 1 (least important) to 5 (most important)			

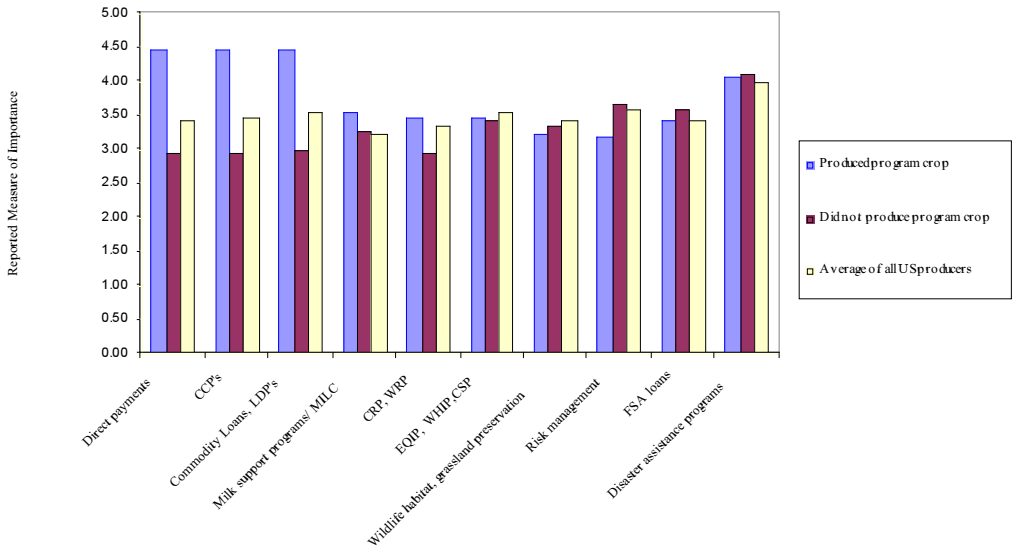

Fig. 1. Importance of maintained funding for current farm programs

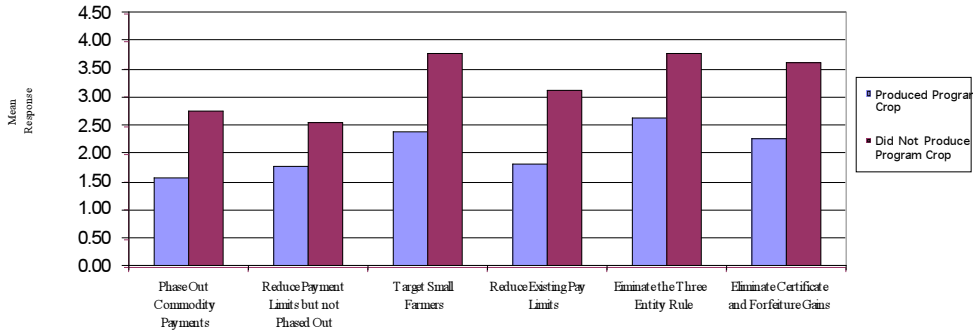

Fig. 2. Comparative responses for program crop participation regarding commodity payments

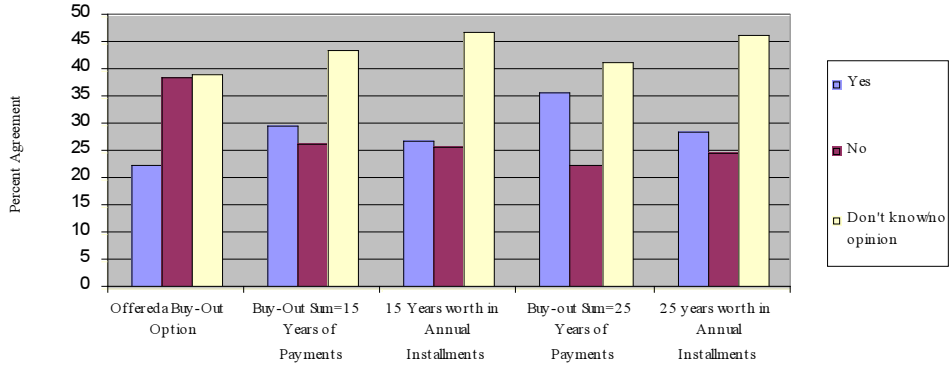

Fig. 3. Support for Commodity Program payment buy-out options, Arkansas producers.

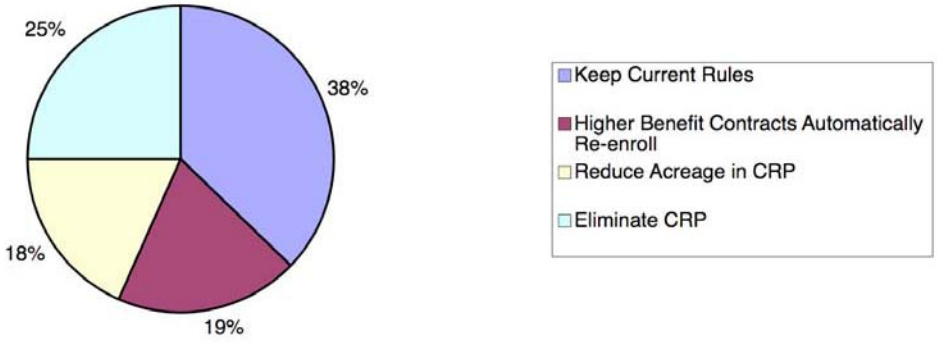

Fig. 4. Conservation Reserve Program

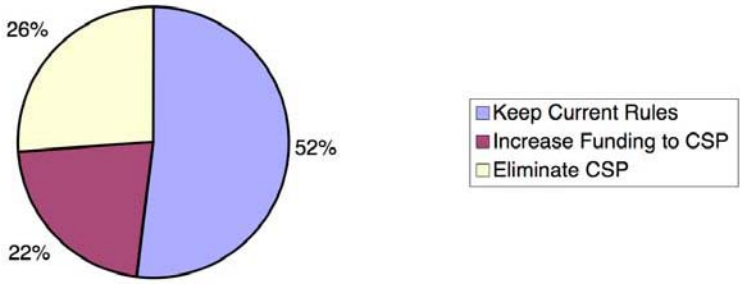

Fig. 5. Conservation Security Program

Mycorrhizal infection rates in RoundupReady® row crops in response to glyphosate and phosphorus applications

Aaron L. Daigh[*], *Mary C. Savin*[†], *and Larry C. Purcell*[§]

ABSTRACT

Currently, the majority of soybean, corn, and cotton crops grown in the U.S. is RoundupReady® (RR) varieties. RR crops are resistant to the active ingredient, glyphosate [N-phosphonomethyl-glycine], in the herbicide Roundup®. RR crops have been genetically modified by the addition of an enzyme found in *Agrobacterium* sp. strain *CP4 EPSPS* that produces an essential protein, involved with aromatic amino-acid production, that is resistant to glyphosate. Glyphosate translocates via phloem from plant leaf tissues to other areas including the root system, and is thus able to affect the rhizosphere microbial community, including mycorrhizae, which are not resistant to glyphosate. A greenhouse experiment was conducted to determine response of mycorrhizal infection and plant nutrients to glyphosate and phosphorus (P) applications to RR soybean, corn, and cotton. Crops were untreated, or treated with glyphosate in low-P soil or in P-fertilized soil, and grown for 6 weeks, after which roots and shoots were harvested and analyzed for mycorrhizal infection and P concentrations. Plant roots were cleared and stained with Trypan Blue dye and analyzed with a dissecting microscope for mycorrhizae on a percent-root basis. Phosphorus had significant positive effects on plant shoot P concentrations for all crops. Mycorrhizal infection rates showed a negative effect in soybean with reduced infection in the P treatment. Glyphosate for all crops and all treatments showed no effect on mycorrhizal infections or plant shoot-P concentrations. Therefore, our results indicate that glyphosate generally may be disregarded in terms of potential detrimental effects on mycorrhizal-plant interactions or plant-P uptake by soybean, corn, and cotton crops in low-P soil.

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[†] Mary C. Savin is the faculty mentor and an associate professor in the Department of Crop, Soil, and Environmental Sciences.

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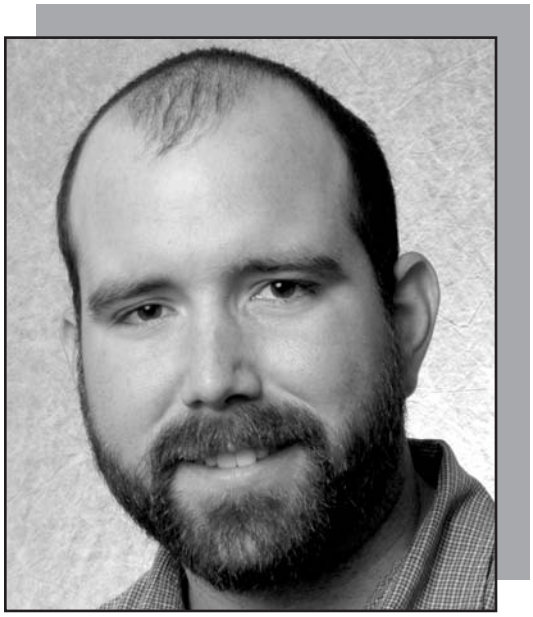
INTRODUCTION

In recent years, since introduction in 1996, the use of RoundupReady® (RR) varieties has increased in popularity and RR cultivars yield the majority of soybean (*Glycine max*), corn (*Zea mays*), and cotton (*Glossypium hirsutum*) crops. In 2002, 72% of U.S. crop hectares consisted of RR crops (USDA-AMS, 2002). RoundupReady® crops are resistant to the active ingredient glyphosate [N-phosphonomethyl-glycine] in the herbicide Roundup® (Monsanto Co., St. Louis, Mo.). Roundup® has gained favor with farmers because of the susceptibility of a broad range of weeds to it and because, being a post-emergence foliar herbicide, there is an increased window of time for application; all of which reduce maintenance, labor, costs, and mixing of numerous herbicides to target multiple weed species, and supports conservative tillage practices (Moschini et al., 2000). Roundup® is also less environmentally persistent because it is degraded relatively quickly once in contact with soil microbes.

Glyphosate targets and inhibits a specific protein and protein derivatives involved in biosynthesis of aromatic amino acids in all plants (Heck et al., 2005). RoundupReady® crops have been genetically modified by the addition of an enzyme found in a strain of *Agrobacterium* sp. *CP4 EPSPS* that produces a glyphosate-resistant transgenic protein, 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS) (Barry et al., 1992; Padgette et al., 1995). Therefore, expression of the resistant EPSPS maintains the aromatic amino-acid biosynthetic pathway, which prevents plant death and preserves crop quality, nutritional health, and yields (Delannay et al., 1995; Hammond et al., 1995; Harrison et al., 1996; Padgette et al., 1995; Nida et al., 1996).

Glyphosate translocates via phloem from plant leaf tissue to other areas including the root system (Hetherington et al., 1999). As a non-selective herbicide, glyphosate is suspected to be detrimental to other living organisms, including microbial communities. The plant-soil interface encompasses increased levels of diverse microbial activity, including mycorrhizae.

MEET THE STUDENT-AUTHOR



Aaron L. Daigh

I am from Fayetteville, Ark., and graduated from Farmington High School in 2003. I enrolled as a chemical engineer major in fall 2003 and became an environmental, soil, and water sciences major in fall 2005. In May 2007 I will graduate with a B.S.A. in environmental, soil, and water sciences and a minor in wildlife habitat management. I served as the Crop, Soil, and Environmental Sciences (CSES) Club president in 2006 and helped the club obtain the Fayetteville Parks and Recreation Volunteer Group of the Year award along with 1st and 2nd place awards for the respective 2005 and 2006 American Society of Agronomy National Club Poster Symposium. I received the Delta Classic Scholarship from CSES, White River Environmental Protection Association Scholarship, James L. Gray Scholarship from the Arkansas Association of Professional Soil Classifiers, and the CSES Outstanding Senior Award.

From fall 2005 until the present, I have worked for Dr. Mary Savin in soil microbiology and ecology. I also worked for Dr. Chuck West in forage science in spring 2006 and Dr. Larry Purcell as part of this project. While working for Dr. Savin and Dr. Purcell, I began research with mycorrhizal responses to glyphosate and phosphorus, and continued into a new research project investigating urea-N mobility in rice soils. I presented research results in the American Society of Agronomy Undergraduate Research Symposium Contest and received a Dale Bumpers College of Agricultural, Food, and Life Sciences Undergraduate Research Grant in fall 2006. I plan to further my education in graduate school with research focused within the soil sciences.

Mycorrhizae are symbiotic fungi involved in intimate exchange of nutrients directly with plant root systems. Mycorrhizae live both within and outside the structure of plant roots. Hyphae of the mycorrhizae extend outward from roots into the soil, functioning similarly to root hairs, as extensions of the root system by increasing surface area and the uptake of water (H_2O) and essential nutrients (particularly phosphorus, P). Studies have shown that adequate P uptake in most of the world's plants is dependent on mycorrhizae-root interaction (Janos, 1980; Hartnett et al., 1993; Koide et al., 1994). Mycorrhizae exchange H_2O and P for carbon in the form of carbohydrates created by photosynthesis within a plant. This intimate relationship between mycorrhizae and the plant generates concern for susceptibility of the fungus to damage attributed to glyphosate application because of the possible translocation of compounds from leaves to the root system.

Currently, over 90% of all soybean crops and increasing numbers of corn and cotton crops within the United States use RR varieties. Due to increased use nationwide of glyphosate-resistant crop varieties, any changes within the rhizosphere communities, particularly to mycorrhizae, could have potential economic consequences. In the event of a detrimental response by mycorrhizal symbionts to glyphosate products, decreased levels of P fertilizer uptake by plant roots are probable and would contribute to P nutrient deficiencies within crops. In the event of P deficiencies following glyphosate applications, farmers would need to apply additional P to fields to maximize plant biomass and crop yield. In the event of no significant detrimental effects to mycorrhizal symbionts after glyphosate applications, then use of conservative P application rates can be considered.

We hypothesized that mycorrhizal sensitivity to glyphosate would decrease mycorrhizal infection in RR crops sprayed with glyphosate. Predictions were that 1) mycorrhizal infection rates will decrease following application of glyphosate to RR row crops, 2) P uptake will decrease from suppression of mycorrhizal infection, and 3) negative effects of glyphosate on mycorrhizae and plant-P nutrition will be overcome by P fertilization.

MATERIALS AND METHODS

The experimental design consisted of a randomized complete block with four treatments in a low-P soil (11 mg P/kg) obtained from a rice field (Hilleman silt loam) in Poinsett County, Ark. Treatments consisted of 1) no added P (0P) and no glyphosate (0Gly) (control); 2) added P as KH_2PO_4 (1P) and 0Gly; 3) 0P with glyphosate (2Gly); and 4) 1P with 2Gly (Table 1). P was added at a rate of 45 kg P/ha, and glyphosate was applied at about 10 and 20 d after emergence at a rate of 1.1 kg/ha. All treatments were used on three plant species: RoundupReady (RR) A4801 soybean, Garst 8553RR corn, and Paymaster 1218RR/GB cotton.

Soil was sieved and placed in 25-cm-diameter pots. A saucer was placed under each pot to retain water and nutrient solutions. Nitrogen (N) fertilizer (as urea) was applied at 112 kg N/ha to corn and cotton pots by placing solution into the saucer and allowing the solution to be taken up by the plants through the bottom of the pots. A symbiotic bacterium, *Bradyrhizobium japonicum* (strain USDA 110) culture, was added to soybean at time of planting seeds to promote optimal nodulation for N_2 fixation. Phosphorus was applied to appropriate treatments in a similar manner as N. Treatments with no added P received 500 ml DI H_2O to keep inputs consistent and maintain similar soil moisture for all pots. Pots were allowed to sit overnight before seeds were planted. Soybean, corn, and cotton seeds were planted at depths of 1.9 cm, 3.8 cm, and 1.3 cm, respectively. For all crops, 4 seeds were initially planted and were thinned to 1 plant per pot before glyphosate application. All pots were watered 2 times per week when the top 2.5 cm of soil was dry.

Glyphosate was applied 10 and 20 days after unfolding of first true leaves for soybean and corn and after the unfolding of the fourth true leaves for corn. Glyphosate was applied at 1.1 kg/ha as Roundup Original Max® in a solution with the aid of a hand-held boom. All plants were allowed to grow for 6 weeks after emergence before harvesting. At harvesting, plant roots were removed manually from the soil, washed with deionized (DI) H_20, and stored in plastic bags on ice until transported to a refrigerator (4°C). Plant shoots were cut and dried at 65°C before being analyzed for P and N. Plant-shoot P was determined by HNO_3 digestion and analyzed on an inductively coupled plasma spectrophotometer (Spectro Analytical Instruments, Fitchburg, Mass.). Total plant-shoot N was determined by the Dumas method with a Leco FP-428 Determinator (Leco Corporation, St. Joseph, Mo.). Plant-shoot P and N were analyzed by the University of Arkansas Soil Testing and Plant Analysis Laboratory, Fayetteville.

For mycorrhizae analysis, a subsample was taken from each plant root system for clearing and staining (Koske and Gemma, 1989). Subsamples were cleared of pigment with 1.8 *M* KOH in test tubes placed in an 80°C water bath for 15 min. Samples were then washed with DI H_20 and soaked in a 3% bleach solution until transparent. Samples were washed again with DI H_20 and dipped in 5 *M* HCl before being placed into a test tube

containing Trypan Blue dye solution. Samples in the dye were then placed into an 80°C water bath for 30 min. Samples were washed and stored in plastic bags in a refrigerator until further analysis.

Infection rates were measured on a percent-root basis with the method used by Giovannetti and Mosse (1980). A 0.50-g portion of each sample was placed in a petri dish containing a grid with 1.27-cm spacing. The sample was spread evenly within the petri dish. A compound microscope was then used to manually count total, infected root intersects over the grid lines. The method allowed determination of percent mycorrhizal infection. Mycorrhizal structures were determined by the presence of hyphae, vesicles, arbuscules, and/or spores (Fig. 1). Significant differences among treatments for each crop species were determined using PROC GLM in SAS with separation of means using least significant differences ($P < 0.05$).

RESULTS AND DISCUSSION

For all treatments and crops, no significant differences were observed for plant nitrogen concentrations, root weights, and shoot biomass (data not shown). Amended P treatments for all crops showed significant increases in plant-shoot P concentrations compared to treatments of no additional P (Fig. 2). However, glyphosate applications did not have a significant impact on plant-shoot P concentrations on any crop species. Increased plant-shoot P concentrations with fertilizer P were greatest for corn, followed by cotton and then soybean. The highest rates of mycorrhizal infection were observed in cotton, followed by corn and then soybean. Mycorrhizal infection rates showed no significant differences for all treatments for corn and cotton (Fig. 3). However, a significant difference was seen in soybean, with a decrease in mycorrhizal infection rate when P was added. Soybean was, therefore, less reliant on mycorrhizae as an aid to acquire P due to adequate uptake by the root system, and therefore showed a decrease in mycorrhizae interaction.

Significant differences in plant-shoot P between 0P soil and the amended-P soil suggest that soil-P was a limiting factor to plant uptake within the experiment. Plant health and ability to carry out normal and vital functions, such as building of DNA and RNA, increase along with increases in P concentrations unless the plant reaches a state of P toxicity (varies among plant species) (Havlin et al., 2005). Glyphosate showed no effect on plant-shoot P concentrations and did not affect the root-mycorrhizal complex's ability to uptake P even in the presence of plant-limiting soil-P.

In conclusion, P fertilization to a low-P soil did have an effect on plant-shoot P concentrations for three species of RR agricultural crops. For mycorrhizal infection rates, P fertilization had a negative effect on RR soybeans only. Glyphosate had no effect for all treatments and all crops tested. Based on our data, we reject the hypothesis that glyphosate will decrease mycorrhizal infection rates. Implications from this study suggest that detrimental effects on mycorrhizal infection rates due to applications of glyphosate to RR soybean, corn, or cotton do not need to be taken into consideration for soil-P recommendations in low-P silt loam soil.

ACKNOWLEDGMENTS

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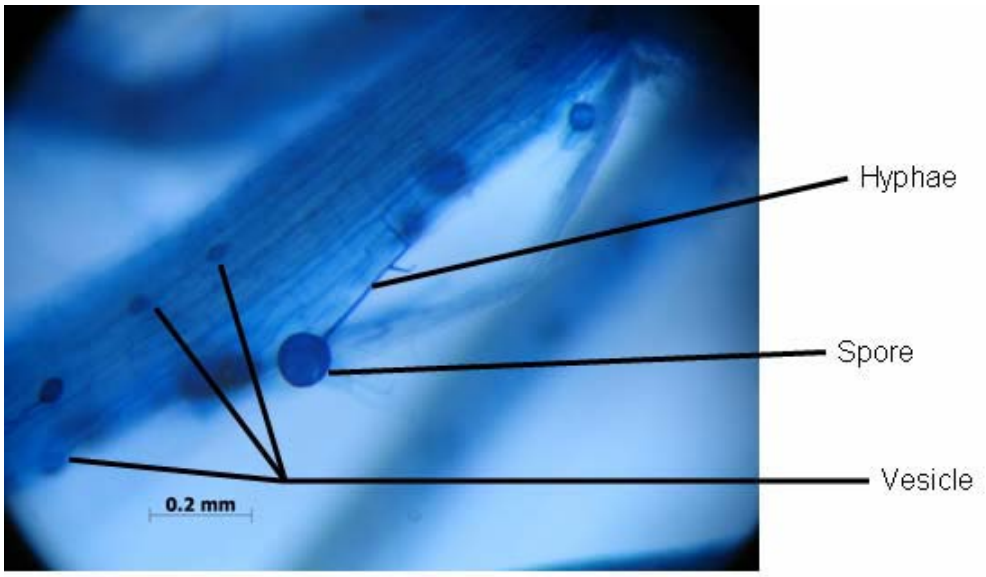

Fig. 1. Mycorrhizal infection of cotton root. Vesicular-arbuscular mycorrhizae can be identified using physical characteristics with the aid of a microscope. Vesicles can be identified as small oval pouches found within the plant root's outer cortex. Spores can be identified as an external circular entity attached to the end of fungal hyphae extending from the root's outer cortex.

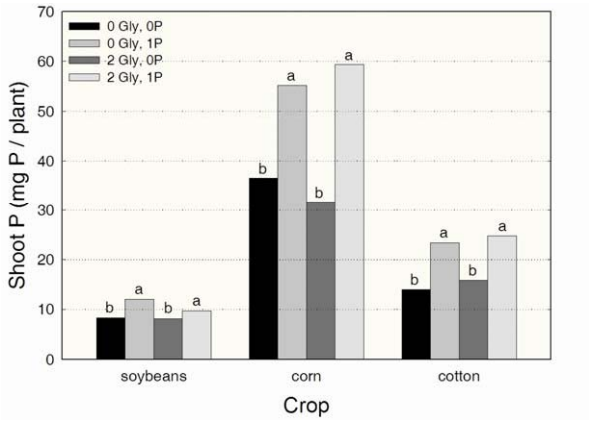

Fig. 2. Soybean, corn, and cotton plant-shoot phosphorus concentration. Treatments included glyphosate (0Gly and 2 Gly) and/or phosphorus (0P and 1P). Different letters within a crop show significant differences, P<0.05.

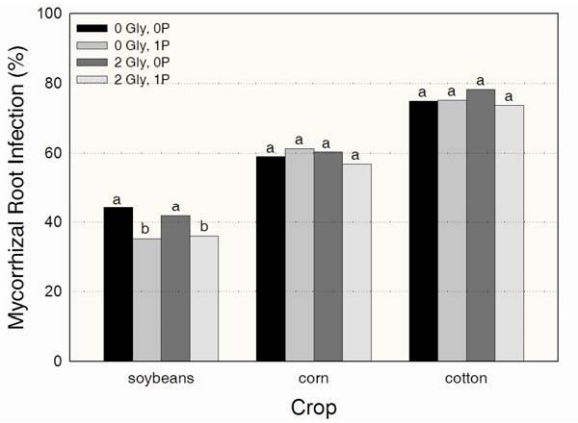

Fig. 3. Effect of treatment (glyphosate (0Gly and 2 Gly) and/or phosphorus (0P and 1P)) on mycorrhizal infection rates of soybean, corn, and cotton roots. Different letters within a crop show significant differences, P<0.05.

Table 1. Glyphosate and phosphorus applied to soybean, corn, and cotton plants

Treatment	Treatment symbols	Glyphosate (kg/ha)	P applied (kg P/ha)
1[z]	0Gly, 0P	0	0
2	0Gly, 1P	0	45
3	2Gly, 0P	1.1 x 2	0
4	2Gly, 1P	1.1 x 2	45

[z]Plants were exposed to 4 treatments: 1) no additional phosphorus (0P) or glyphosate (0Gly), 2) phosphorus (1P) and 0Gly, 3) 0P and glyphosate (2Gly), and 4) 1P and 2Gly. Glyphosate was applied twice at a rate of 1.1 kg/ha at about 10 and again at 20 days

PTO performance and NO_x emissions with D2, B20, and B100 fuels in a John Deere 3203 compact tractor

Matthew K. Hardin[*]*, Tonya Brown*[†]*, Melanie R. Roller*[§]*, Donald Johnson*[‡]*, and George Wardlow*[§§]

ABSTRACT

Tests were conducted in fall 2006 on a John Deere 3203 diesel tractor to determine differences in specific fuel consumption, power take-off (PTO) torque, PTO power, thermal efficiency, and oxides of nitrogen (NO_x) emissions between No. 2 diesel (D2), 20% biodiesel (B20), and 100% biodiesel (B100). Four 1-hour tests were conducted on each fuel. The results indicated no statistically significant differences ($p \leq .05$) between D2 or B20 on any variable of interest. However, B100 resulted in significantly ($p \leq .05$) increased, specific fuel consumption and thermal efficiency and decreased PTO torque and PTO power over both D2 and B20. These data suggest that farmers could switch from D2 to B20 without any performance losses, but a switch to B100 would result in the use of more fuel and a loss of power and torque.

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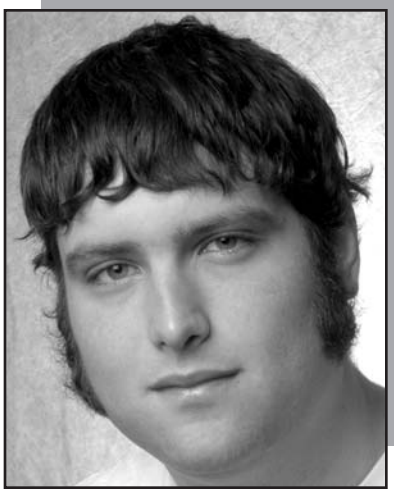
[†] Tonya Brown is a senior agricultural education, communication and technology major.

[§] Melanie Roller is a senior agricultural education, communication and technology major.

[‡] Donald Johnson is a professor in the Department of Agricultural and Extension Education.

[§§] George Wardlow is a professor and interim department head of the Department of Agricultural and Extension Education .

MEET THE STUDENT-AUTHORS



Matthew K. Hardin

I came to the University of Arkansas from Conway, Ark., in 2003 to major in journalism. By spring 2004, I found a home in the agricultural communications program of the Agricultural Education, Communication and Technology program of the Bumpers College. I have been active on campus in the Agricultural Communicators of Tomorrow and as a resident assistant. I completed an internship with the Arkansas Farm Bureau in Little Rock in the area of communications technologies, during which I further developed my photographic skills. I graduated with a bachelor of science degree in agriculture in spring 2007.

Tonya Brown

I graduated from high school in Tecumseh, Okla., in 2002, having been very active in FFA and 4-H. I completed my bachelor of science in fall 2006 with a major in agricultural education, communication and technology. During my program I was a member of the livestock judging team and was active in Collegiate FFA/4-H. I served as president of the Collegiate Farm Bureau. I plan to teach agricultural sciences and technologies at the high-school level in Oklahoma or Arkansas.

Melanie R. Roller

I completed high school in Pea Ridge, Ark., in 2003, where I was very active in several student organizations, including the FFA. After coming to the University of Arkansas, I continued my participation in Collegiate FFA/4-H, serving in several officer roles. I was also an officer in the Collegiate Farm Bureau. I plan to graduate summer, 2007 with a bachelor of science in agriculture and a major in agricultural education, communication and technology. Beginning in fall 2007, I will be employed as an agricultural science teacher for the McDonald County Schools in Anderson, Mo.

INTRODUCTION

Given the recent rise in cost of petroleum-based fuels and the continuing U.S. dependency on them, the agricultural equipment industry is producing more tractors that can run on alternative fuels (Cousins, 2006). These fuels need to be studied to determine if they are viable alternatives to fossil fuels.

The National Biodiesel Board (NBB, 2007) defines biodiesel as a "fuel comprised of mono-alkyl esters of long-chain fatty acids derived from vegetable oils or animal fats, designated B100, and meeting the requirements of ASTM D 6751." The natural oil can be vegetable oils, cooking greases and oils, or animal fats (DOE, 2006). Most U.S. biodiesel is produced from the methyl ester of soybean oil, since this crop is available in sufficient quantities on a national level (Canakci and Van Gerpen, 2003).

Researchers (Proc, et al., 2006; Canakci and Van Gerpen, 2003; Schumacher et al., 2001) found little difference in power performance, specific fuel consumption, or thermal efficiency between engines fueled with No. 2 petroleum diesel (D2) or with blends of 80% D2 and 20% biofuel (B20). A study at Iowa State University found that biodiesel blends were similar to D2 in their thermal efficiency, but had higher fuel consumption (Monyem and Van Gerpen, 2000). Biodiesel was found to produce 15% and 16% lower exhaust carbon monoxide and hydrocarbons, respectively, than fossil fuels but there was no difference between the NO_x and smoke emissions of D2 and biodiesel (Monyem and Van Gerpen, 2000).

A University of West Virginia study used a 35% biodiesel blend on heavy-load engines. They found specific fuel consumption to be about the same as with D2 (Wang et al., 2000). Heavy trucks emitted lower particulate matter, carbon monoxide, and hydrocarbons than the same trucks fueled with D2 (Wang et al., 2000). Decreased power and increased specific fuel consumption have been found in engines fueled with B100, since it contains approximately 13% less energy than D2 (DOE, 2006).

One of the main arguments for the use of biodiesels is that they are better for the environment, but some researchers dispute this fact. Oxides of nitrogen (NO_x) are an EPA-regulated pollutant. Some researchers found increased NO_x emissions with biofueled engines (Canakci and Van Gerpen, 2003; Schumacher et al., 2001), while others (Proc et al., 2006) found no increase. Ongoing tests at the National Renewable Energy Laboratory show that "NO_x emissions do not always increase with B20 and in some cases actually decrease" (DOE, 2006).

The goal of this study was to determine if there were significant differences between PTO power, PTO-specific fuel consumption, PTO thermal efficiency, or PTO-specific NO_X emissions for a compact utility tractor fueled with D2, B20, or B100.

MATERIALS AND METHODS

Four one-hour steady-state tests were conducted using each fuel (D2, B20, and B100). PTO speed was maintained at a steady 540 rpm. PTO rpm, torque, power, mass fuel consumption, and NO_X emissions were measured at five-minute intervals. Ambient environmental conditions were monitored to ensure the tests were in compliance with the OECD Tractor Test Codes (OECD, 2006). The tests were conducted on a John Deere 3203 compact tractor with approximately 900 hours of prior machine use. It had a Yanmar three-cylinder diesel engine (Table 1) with gross engine power rated at 23.9kW (Compact Utility Tractor 3203 Operator's Manual, n.d.). The fuel system was drained and the oil filter was replaced prior to testing each fuel. An auxiliary fuel tank and an Ohaus SD-35 digital platform scale (35kg x 0.02kg) were used to measure the mass fuel consumption. An AW NEB 400 PTO dynamometer was used to apply the load and measure PTO performance (AW Dynamometer, Colfax, Ill.). The NO_x emissions were measured with an Auto Logic Gold 6-Gas exhaust analyzer (Auto Logic, Sussex, Wis.). The general characteristics of the fuels used are reported in Table 2.

RESULTS AND DISCUSSION

There was no significant difference in mean PTO speed by fuel type, with all mean speeds within 0.16% of the target speed (Table 3). There were no significant differences ($p \leq .05$) between D2 and B20 on any measure: PTO power, PTO torque, fuel consumption, NO_x emissions, or PTO rpm. When fueled with B100, the tractor produced significantly less PTO power and torque than when fueled with D2 or B20.

PTO-specific fuel consumption was significantly higher ($p \leq .05$) for B100 than for D2 or B20. However, PTO thermal efficiency was significantly ($p \leq .05$) higher for B100 than for D2 or B20. Finally, there was no significant difference ($p \leq .05$) in PTO-specific NO_x emissions between D2, B20, or B100.

The results related to power, torque, and specific fuel consumption support the findings of previous studies (Canakci and Van Gerpen, 2003; Schumacher et al., 2001). However, results related to thermal efficiency and NO_x emissions differ from previous studies. The lack of

statistically significant differences in NO_x emissions across the three fuels, which are different results from previous studies, may result from differences between the testing methods used. Canakci and Von Gerpen (2003) tested under full-load conditions, while Schumacher et al. (2001) tested under transient-load conditions. Tests reported herein were conducted at rated PTO speed, which is a light load, but at high engine speed. NO_x emissions have been shown to increase with increased load and decreased engine speed (Li et al., 2006; DOE, 2006). The slightly higher thermal efficiency with B100 is likely due to load conditions and the energy content of the specific fuels used in this study.

These results confirm that tractors similar to this one may be fueled with either D2 or B20 with no significant differences in performance or specific fuel consumption. Fueling with B100 will result in increased PTO-specific fuel consumption, with a decrease in power and torque. When fueled with B100, the tractor PTO thermal efficiency was slightly higher than with D2 or B20.

Farmers can use these data to decide if they should switch to biodiesel or should continue to use D2. If the price of B20 is less than D2, and a farmer can use B20 without any performance losses, the conversion to B20 makes economic sense. However, the use of B100 would result in performance loses.

Further testing should be conducted to determine if there is a significant difference in NO_x emissions at increased load and decreased engine speed. Full-load testing would also allow for further evaluation of the higher PTO thermal efficiency found for B100.

In this study, the researchers did not consider the potential differences in engine wear, fuel system degradation, or cold-start issues associated with the use of biofuels. Consumers should take these factors into consideration, consult the manufacturers' warranty conditions, and follow the recommendations when selecting fuels.

ACKNOWLEDGMENTS

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Table 1. John Deere 3203 specifications

Engine	Yanmar
Bore	84 mm
Stroke	90 mm
Displacement	1.5L
Gross engine power (rated)	23.9 kW
Compression ratio	19:01

[a]Compact Utility Tractor 3203 Operator's Manual

Table 2. Specific fuel characteristics

Property	D2	B20	B100
Carbon (% mass)	84.42[a]	85.10[a]	77.30[a]
Hydrogen (% mass)	13.38[a]	12.60[a]	11.80[a]
Specific gravity	0.814[a]	0.862[c]	0.886[a]
Kinematic viscosity, 40 oC (mm2/s)	N/A	2.92[a]	4.12[a]
Cetane number	46.1[a]	46.0[a]	47.5[a]
Heat of combustion, gross (BTU/lb)	19832[b]	19253[c]	16937[b]

[a]Values from DOE National Renewable Energy Laboratory Fuels Database.

[b]Analysis by Magellan Testing Laboratory (Kansas City, Kan.).

[c]Calculated value.

Table 3. Mean data for each output

	N		PTO RPM	PTO power (KW)	PTO torque (N-m)	Thermal efficiency (%)	SFC (kg/kW-h)	NOx emissions (g/kW-h)
D2	4	$\bar{x}$	540.15[a]	17.32[a]	306.48[a]	23.9[a]	0.327[a]	6.00[a]
		S.D.	0.0896	0.2880	5.6810	0.0040	0.0055	0.278
B20	4	$\bar{x}$	540.82[a]	17.19[a]	303.06[a]	24.4[a]	0.33[a]	6.23[a]
		S.D.	0.7500	0.0431	1.3843	0.0035	0.0047	0.073
B100	4	$\bar{x}$	540.25[a]	15.96[b]	282.25[b]	25.00[b]	0.366[b]	6.16[a]
		S.D.	0.1732	0.2358	4.7374	0.0065	0.0093	0.319
		F	1.14	48.19	36.45	5.44	40.17	0.087
		p	0.3617	< .0001	< 0.0001	0.0283	< 0.0001	0.4524

[a,b] Means with the same letters within columns are not significantly different.

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 ($p = .2748$).

[*] Garris Hudson graduated in May, 2007 with a B.S. in agricultural education, communication, and technology.

[†] 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.

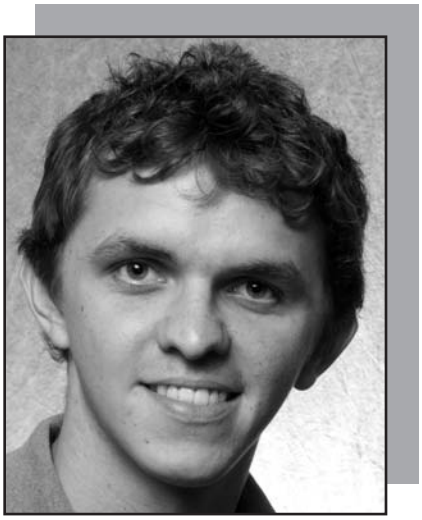
[‡] Donald Johnson is a professor of agricultural systems technology management in the Department of Agricultural and Extension Education.

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.

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.



Robby Shofner

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 (Grisso 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 (Grisso and Alley, 2002).

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.

MATERIALS AND METHODS

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 (m_j), root mean square (rms_j) error, and standard deviation of error (std_j) were calculated using the following equations:

$$m_j = \frac{1}{N}\sum_{i=1}^{N} ij$$

$$rms_j = \sqrt{\frac{1}{N}\sum_{i=1}^{N} e_{ij}^2}$$

$$std_j = \sqrt{\frac{1}{N}\sum_{i-1}^{N} \left(e_{ij} - m_j\right)^2}$$

Where,

N = the number of data points obtained per swath (6)

e_{ij} = 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 overcorrect; 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.

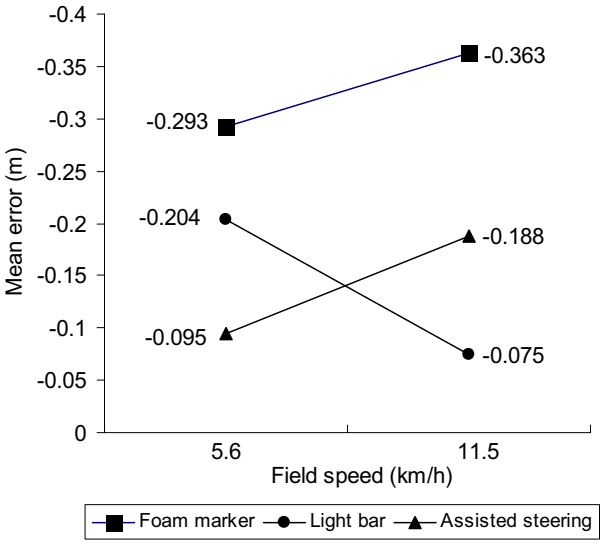


Fig. 3. Mean error by guidance method and field speed.

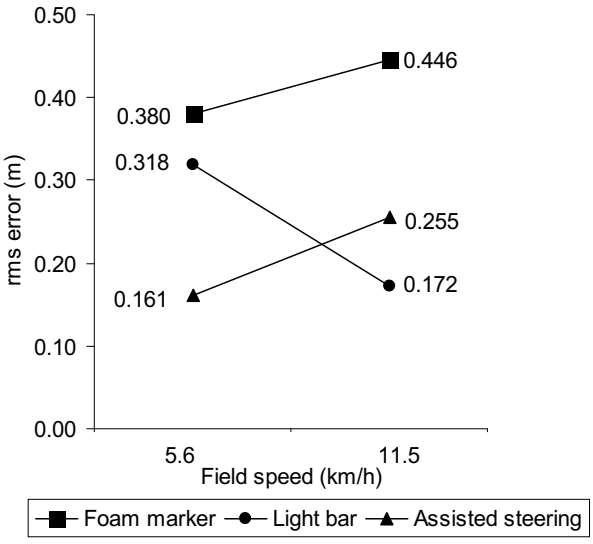


Fig. 4. RMS error by guidance method and field speed.

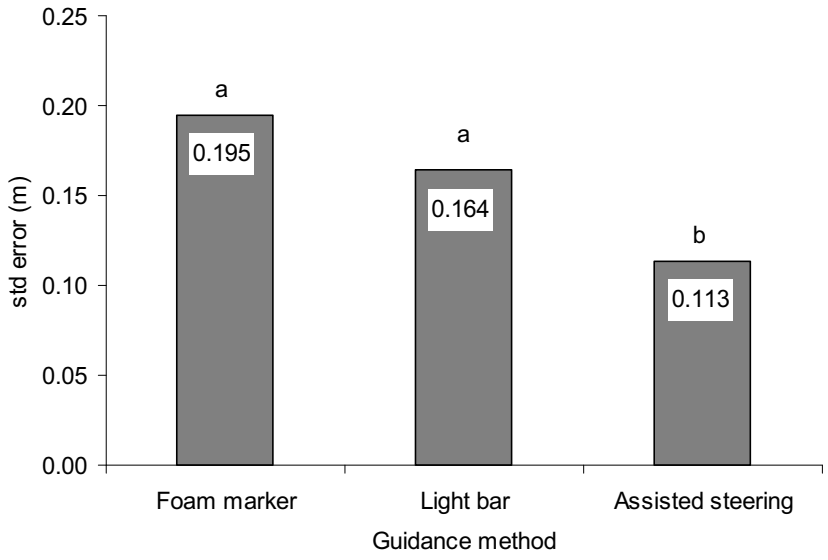


Fig. 5. STD of error by guidance method and speed. Bars with different letters are significantly different.

A computational model for analysis of uncoupled NO synthase on nitric oxide and superoxide interaction in microcirculation

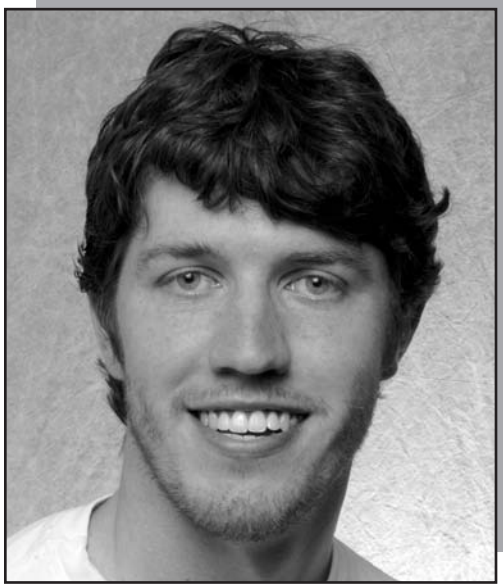
William J Richardson[*] *and Mahendra Kavdia*[†]

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.

[*] William Richardson is a senior majoring in biological engineering.

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



William J. Richardson

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.

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

peroxynitrite, along with molecules such as SOD and CO_2, 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 O_2^- 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 O_2^- 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, O_2^-, 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, O_2^- generation is included as an overall rate of production. Peroxynitrite is produced only by reaction of NO and O_2^- and is considered to occur in all regions.

In deriving mass balance of the three species (NO, O_2^-, 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,

$$D_j \nabla^2 C_j \pm \sum R_{j,i} = 0 \qquad (1)$$

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 (ONOOH) (Nalwaya and Deen, 2003). ONOOH is in acid-base equilibrium with $ONOO^-$.

Boundary Conditions. Continuities of NO, O_2^-, 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 O_2^- were given by Equations 2a and 2b.

$$Q_j = D_j \frac{\partial C_{j,cf}}{\partial r} - D_j \frac{\partial C_{j,en}}{\partial r} \qquad (2a)$$

$$Q_j = D_j \frac{\partial C_{j,cf}}{\partial r} - D_j \frac{\partial C_{j,en}}{\partial r} \qquad (2b)$$

where $_j$ stands for NO and O_2^-, and Qj 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-

cle 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 (Boegehold, 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/ $(1+10^{pK_{per}-pH})$ 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

multiple (or all) of the four endothelial points, which included the left side of arteriole, right side of arteriole, left side of venule, and right side of venule. In the case of only venular uncoupling, values of these four points were decreased by 0, 6.9, 53, and 56%, from left to right. In the case of both venular and arteriolar uncoupling, these changes became 55, 64, 80, and 74%, from left to right.

Superoxide profiles for both cases were very similar to the base case profiles, varying only in height of concentration peaks, which were all located in the endothelial regions. Uncoupling of the venule produced small increases of 0, 1.2, 7.0, and 6.9%, from left to right. In the case of both the arteriole and venule being uncoupled, changes were greater, equaling +10, +12, -60, and -61%, from left to right. Concentration profiles for peroxynitrite follow the same general curve as the base case but again values at the four endothelial points are decreased. For just the uncoupled venule, changes in concentration from the base case equal 0, -6.8, -42, and -46%, from left to right. With uncoupling occurring in both the venule and the arteriole, changes equal -45, -56, -84, and -81%, from left to right.

Discussion of uncoupled eNOS. There was a significant decrease of NO concentration values in the cases of uncoupled NOS. In the case of both arteriolar and venular uncoupling, the concentration profile never exceeded that of the steady-state value reached in the parenchymal tissue. This argues that heightened superoxide levels act to consume the majority of available NO across all regions of the vasculature. Only in the distant tissue was an effect not seen. It is very significant that when only the venular eNOS is uncoupled, a NO decrease of 6.9% was seen in the arteriole endothelium. As literature has shown the ability of venular produced NO to increase arteriole NO concentrations (Guzik et al., 2002; Kavdia, 2006), our model suggests that superoxide produced in the venule can indirectly affect the neighboring arteriole and decrease NO concentrations in the arteriolar endothelium. Thus, venular endothelial dysfunction can impair not just venular dilation but arteriolar dilation as well.

Importance and conclusion. The model has shown that the uncoupling of eNOS can be a significant factor in endothelial dysfunction and reduced NO. One aspect of endothelial dysfunction that was not included in this model was the percentage of eNOS that was uncoupled. BH_4, the cofactor of eNOS, can be oxidized by both superoxide and peroxynitrite (Li and Shah, 2004) and results in uncoupled eNOS. Our model simulation of uncoupled eNOS considered all nitric oxide synthase to be uncoupled but in actual human vasculature, the 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 all regions. This is especially significant to consider since the case of venular uncoupling reduced NO 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^-, $ONOO^-$, 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.

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Table 1: Model Parameters

Parameter	*Value*	*Units*	*Reference*
Systemic Hematocrit	45	%	Text
Capillary Hematocrit	30	%	Text
O_2 concentration	27	μM	Popel, 1989
SOD concentration	1 (0.1)	μM	Buerk et al., 2003
CO_2 concentration	1.14 (0.114)	mM	
Q_{NO}, half of NO release	2.65×10^{-12}	mol/cm^2-s	Vaughn et al., 1998
Q_{sup}, half of O_2^- release	0.2 (2) x Q_{NO}	mol/cm^2-s	Text
D_{NO}	3.3×10^{-5}	cm^2/s	Zacharia and Deen, 2005
D_{sup}	2.8×10^{-5}	cm^2/s	Nalwaya and Deen, 2003
D_{per}	2.6×10^{-5}	cm^2/s	Nalwaya and Deen, 2003
f in tissue	0.640		Text
f in blood	0.817		Text
Uncoupled eNOS NO release	1/3 x Q_{NO}	mol/cm^2-s	Shinozaki et al., 1999
Uncoupled eNOS O_2^- release	3 x Q_{sup}	mol/cm^2-s	Vasquez-Vivar et al., 1998
Geometry:			
Arteriole radius	25	μm	Text
Venule radius	50	μm	Text
Distance between centers	100	μm	Text
Art Cell Free thickness	4.5	μm	Rojas et al., 2006
Ven Cell Free thickness	2.0	μm	
Endothelium thickness	0.5	μm	Wood et al., 2006
Interstitial Space thickness	0.5	μm	Kavdia et al., 2002
Smooth Muscle thickness	6.0	μm	Haas and Duling, 1997
NPT thickness	5.0	μm	Kavdia and Popel, 2006
NO reaction rates:			
NO + O_2 : k_{O2} $-k_{O2}C_{NO}^2C_{O2}$	9.6×10^6	M^{-2}s^{-1}	Lewis and Deen, 1994
NO + O_2^- : k_{perp} $- k_{perp}C_{NO}C_{O2-}$	6.7×10^9	M^{-1}s^{-1}	Huie and Padmaja, 1993
NO + sGC : k_{sm} $- k_{sm}C_{NO}^2$	5×10^4	M^{-1}s^{-1}	Vaughn et al., 1998
NO + RBC	1.4×10^5	M^{-1}s^{-1}	Carlsen and Comroe, 1958
NO + RBC in CR: k_{cr} $- k_{cr}C_{NO}$	1270	s^{-1}	Text
NO in capillaries: k_{cap}	12.4	M^{-1}s^{-1}	Text

$NO + ONOO^- : k_{per}$ $-k_{per}C_{NO}fC_{per}$	9.1×10^4	$M^{-1}s^{-1}$	Pfeiffer et al., 1997
O_2^- reaction rates:			
$O_2^- + SOD: k_{SOD}$ $-k_{SOD}C_{O2}\text{-}C_{SOD}$	1.6×10^9	$M^{-1}s^{-1}$	Fridovich, 1995
$ONOO^-$ reaction rates:			
$ONOO^- + CO_2 : k_{CO2}$ $-k_{CO2}C_{CO2}fC_{per}$	5.6×10^4	$M^{-1}s^{-1}$	Radi, 1998

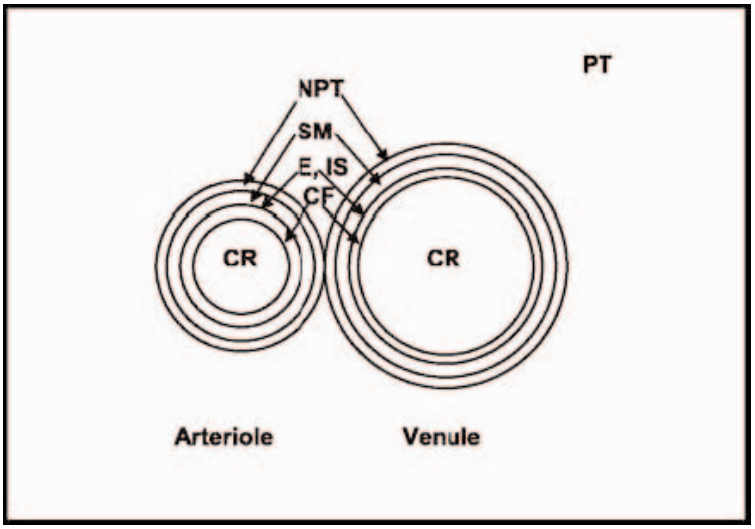

Fig. 1. Model Geometry
The model area includes the arteriole and venule pair, surrounded by parenchymal tissue (PT) containing capillaries. Both the arteriole and venule have a cell-rich (CR) and cell-free (CF) region referring to the presence or absence of red blood cells in the lumen; endothelium (E); 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).

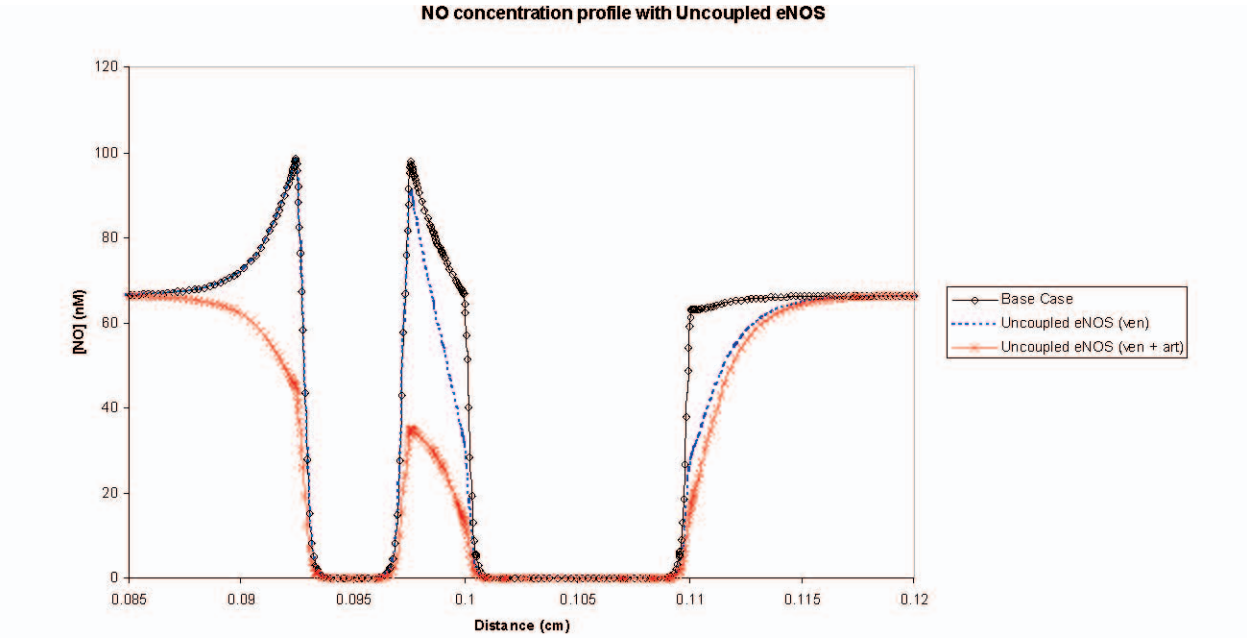

Fig. 2. Effect of uncoupled eNOS on NO profile. To investigate the effect of uncoupled nitric oxide synthase on the species profiles, the parameters for endothelial NO and superoxide production were altered. For the simulation, 1/3 x normal NO release and 3 x normal superoxide release were used in two cases: a) just the venular endothelium, and b) both venular and arteriolar endothelium.

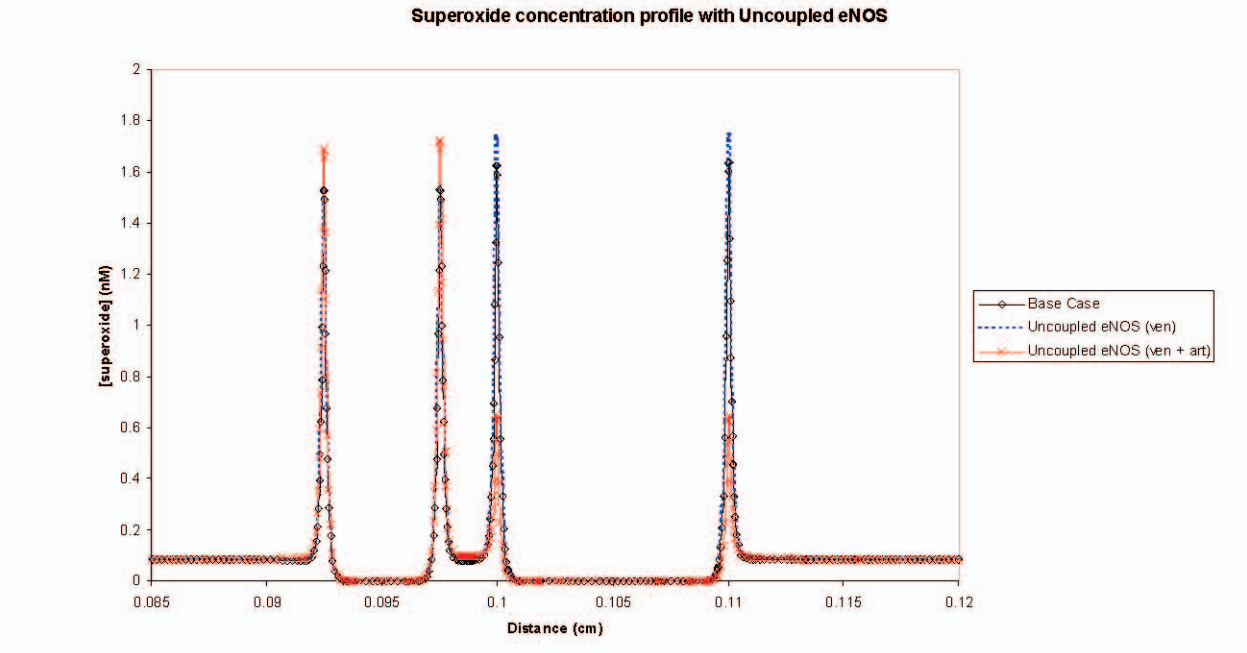

Fig. 3. Effect of uncoupled eNOS on O_2^- profile.

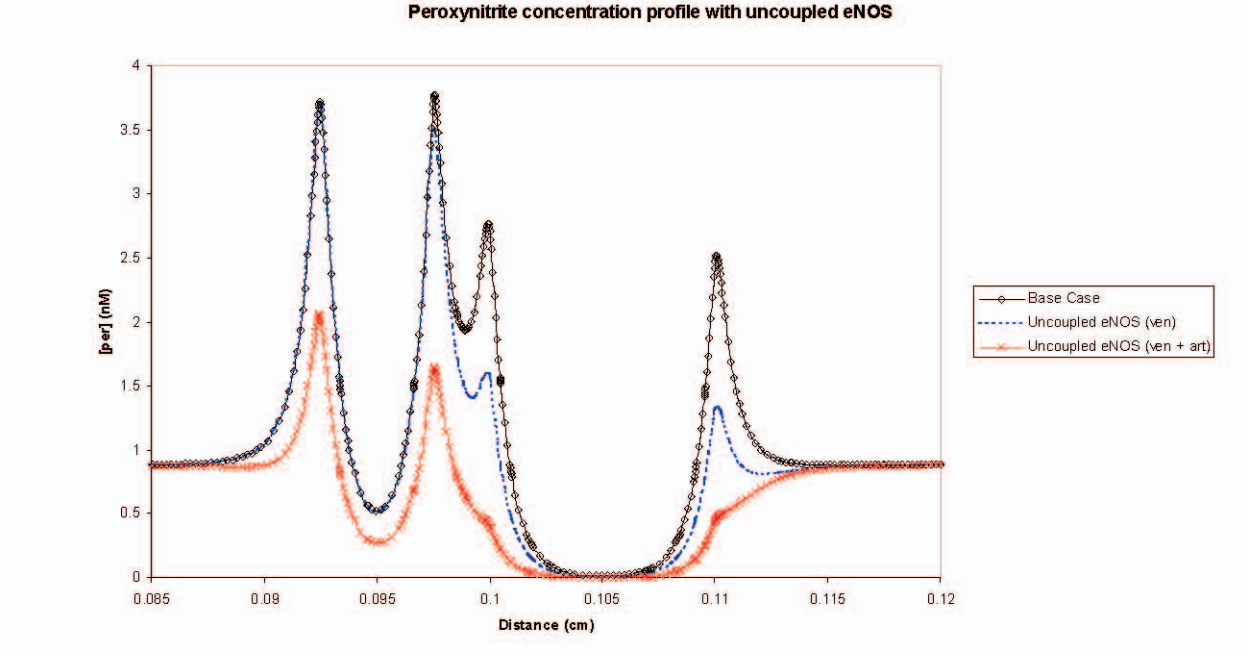

Fig. 4. Effect of uncoupled eNOS on $ONOO^-$ profile.

Comparison of instrumental methods for measuring seed hardness of food-grade soybean

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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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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

Mioko Tamura

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.

experimental errors when using single samples. The major disadvantages of shear test are 1) no uniform protocol, and 2) multiple variable sources involved in the sample preparation and testing processes. A compression test is rarely used because gases are easily trapped in most products (Wheeler, 1994). There is no standard method in testing soybean seed hardness for natto manufacturing.

The objectives of this research were 1) to evaluate five food-grade soybean genotypes for seed-swell ratio and hardness, 2) to determine the optimal amount of steamed seeds required to quantify seed hardness for each testing, and 3) to examine the relationship between the seed-swell ratio and hardness and compare different hardness-testing methods.

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 blot-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/cm^2. Sub-samples from each genotype were taken, as appropriate, for each type of hardness 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 caliber. 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 $P \leq 0.05$ 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 (R^2) 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

(2.30) than did V97-6490. In Mullin and Xu's (2001) study, OX 591, with a similar seed size (15.6 g/100 seeds) to Hutcheson, had a swell ratio by weight of 1.54 that was much lower than that of Hutcheson, because OX 591 was a hard-seeded line with 72.4% non-water-absorption seeds. The swell ratio by weight ranged from 2.36 to 2.51 among 16 Iowa small-seeded lines (Geater et al., 2000), which was higher than that of MFS-591 and Camp, two small-seeded soybeans in our study. Apparently, genotype affected the swell ratio significantly. Despite the seed-size difference, all genotypes except for MFL-552 exhibited similar seed-size dimension change before and after soaking. Therefore, the swell ratio by volume, weight, and dimension did not show the same trend on these five genotypes.

The correlation of seed size and swell ratio as well as correlation between swell ratios is shown in Table 2. Swell ratio by weight (WE) and volume (VO) had the highest correlation coefficient of 0.81 at $p < 0.0001$ level. Swell ratios determined by both volume and weight are more reliable than those determined by dimension. However, using seed-weight change to determine swell ratio is much easier than using volume of water absorbed or seed-dimension measurement. Seed size (SS) was significantly and negatively correlated with both swell ratio by weight (WE) and swell ratio by volume (VO), with negative correlation coefficients of –0.51 and –0.32, respectively. The correlation coefficient between seed size and swell ratio by weight was –0.81 in Mullin and Xu's study (2001). The possible reason for the lower correlation coefficient obtained in this study was that we tested the soybean genotypes with a wider seed-size range (6.8 to 38.1 g/100 seeds) than those in Millin and Xu's study (15.6 to 36.3 g/100 seeds). In addition, the specific genotypes and number of genotypes tested may have influenced the correlation coefficient. Swell ratio by seed dimension had insignificantly positive correlation with seed size and with swell ratio by weight and volume. Therefore, seed-dimension change before and after soaking might not serve as a good indicator for water absorption.

Seed hardness measured by five different methods.

The seed hardness determined by each testing procedure is given in Table 1. Seed hardness by single-blade, cylinder, and shear-cell probes showed a linear increase with increased sample size. For the single-blade procedure, the hardness of five seeds was about five times higher than the hardness of one seed. For the cylinder procedure, the hardness of one, five, and ten seeds did not show the exact proportional increase as the sample sizes increased, except for the hardness of five and ten MFS-591 seeds (25.3 N and 51.7 N, respectively). Increase of hardness was also linear but not exactly proportionate 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 rigs 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 rigs 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.

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Table 1. Seed physical traits and hardness of five food-grade soybeans.

Genotype	Seed size	Swell ratio			PB†	SB†		
	(g/100sd)	volume	weight	dimension	1 seed	1 seed	5 seeds	LSD
V97-6490	38.1	2.2A‡	2.4A	2.8A	3.4A	4.3bA	27.5a§A	1.6
MFL-552	21.8	2.0B	2.3B	1.9B	1.6B	3.5bB	15.2aB	1.0
Hutcheson	15.4	1.8C	2.3C	2.5A	0.8C	2.2bC	8.5aC	0.6
MFS-591	8.7	2.0B	2.3B	2.5A	1.4B	1.8bC	8.5aC	0.7
Camp	6.8	2.0B	2.3B	2.7A	1.1BC	2.2b	8.4aC	0.6
LSD		0.08	0.03	0.43	0.55		1.22	

	CY†				PR†	SC†				
Genotype	1 seed	5 seeds	10 seeds	LSD	16 seeds	20 g	30 g	40 g	50 g	LSD
V97-6490	31.1cA	99.1bA	152.5aA	14.5	72.6B	485c	800bA	970ab	1049a	207.0
MFL-552	15.5cB	48.3bB	69.6aB	7.5	26.8D	303d	422cC	523b	606a	69.8
Hutcheson	17.4cB	39.1bB	63.6aBC	6.0	57.8B	391c	649bB	760b	1189a	156.2
MFS-591	10.5cB	25.3bC	51.7aC	5.8	34.1C	348d	483cC	534b	703a	45.0
Camp	19.2c	40.0b	61.6aBC	4.5	54.2A	491c	670bB	1089a	1086a	129.5
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 2. Correlation among seed swell ratios and seed size of five food-grade soybeans as measured by different methods.

Traits	VO	WE	DI
SS	-0.32*	-0.51**	0.11
VO		0.81***	0.16
WE			0.14

*, **, ***: 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.

No. of	CY†			PB†	SB†		PR†	SC†			
replication	1 seed	5 seeds	10 seeds	1 seed	1 seed	5 seeds	16 seeds	20g	30g	40g	50g
5	0.37Ac‡	0.20bA	0.14a§A	0.35A	0.30bA	0.11aA	0.09A	0.21aA	0.14aA	018aA	0.19aA
10	0.44Ac	0.20Ab	0.15Aa	0.32A	0.34Ab	0.13Aa	0.10A	0.21Aa	0.16Aa	0.18Aa	0.17Aa
15	0.44Ac	0.20Ab	0.15Aa	0.31A	0.31Ab	0.13Aa	0.11A	—	—	—	—
Mean	0.42	0.20	0.15	0.33	0.32	0.12	0.10	0.21	0.15	0.18	0.18

†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.

Methods‡	CY	PB	PR	SB	SC
CY		0.54***	0.09	0.82***	0.32*
PB			-0.09	0.67***	0.15
PR				-0.13	0.53***
SB					0.32*

‡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 $p \leq 0.05$, 0.01, and 0.0001 levels, respectively.

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

Traits‡	SB§			CY§			SC§		
	R^2	Intercept	Slope	R^2	Intercept	Slope	R^2	Intercept	Slope
SS	0.70	27.14	-4.51	0.45	136.35	-19.14	0.02	663.92	-19.76
VO	0.41	-53	33.16	0.30	-252.93	166.11	0.02	281.76	161.28
WT	0.62	-192.96	89.32	0.53	-1029.43	480.11	0.06	-913.59	657.24
DI	0.01	10.94	0.98	0.01	55.75	9.67	0.25	202.77	162.7

‡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.

Cylinder compression | 2-mm probe puncture | Single blade shear | shear cell shear | Pea rigs puncture

Fig. 1. Five probes used in testing seed hardness of cooked soybean.

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