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Several Authors

DISCOVERY

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Vol. 17 Fall 2016

UofA DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

UNIVERSITY OF ARKANSAS
DALE BUMPERS COLLEGE
OF AGRICULTURAL, FOOD
& LIFE SCIENCES

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Vol. 17 **Fall 2016**

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Cover: Lauren Cambias works with her faculty mentor, Jamie Baum collecting data for her project, Evaluation of protein source at breakfast on energy metabolism, metabolic health, and food intake: a pilot study. Photo by Fred Miller.

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Undergraduate Research Articles *continued*

Letter from the Dean

Proudly Presenting Life-Changing Work and Research Experiences of Our Students

Lona Robertson

The *Discovery* undergraduate journal promotes the abilities of our students by highlighting work and research they have completed inside and outside the classroom with the help of our outstanding faculty in a citable publication.

Completing these projects helps prepare our students for professional careers in the areas of food, agriculture, the environment, and human quality of life.

Projects can be designed to meet requirements for an honors thesis in the Bumpers College Honors Program and some have been funded by our Undergraduate Creative Projects/Research Grants Program.

This issue provides studies from different departments and concentrations within the Bumpers College.

Inside, you will discover:

- the impact and role of women in the state's agricultural industry over the last 10 years
- the effect of shade on fruiting blackberries in a controlled environment
- how protein for breakfast impacts energy metabolism, metabolic health and food intake
- how precision field data can be used for more efficient combine harvesting
- the results of a comparison growth study on goats fed calcium from different sources
- an evaluation of harvest and storage temperatures on the firmness and incidence of red drupelet reversion development of blackberries
- an evaluation of a streambank restoration project at Fayetteville's Botanical Garden of the Ozarks
- studies on fertility in beef heifers, the use of the nuclease I-*Sce*I in excising selectable marker genes from plant genomes and the status of the Northern Saw-whet owl in Arkansas

Congratulations to the student authors, and thank you to the faculty mentors and editors who made this research and this year's journal possible. We are pleased and proud to present this work as a service to you and all our readers.

Lona Robertson, Interim Dean
Dale Bumpers College of Agricultural, Food and Life Sciences

A Message from the Faculty Editor

Continuing the Tradition of Excellence from Bumpers College Students

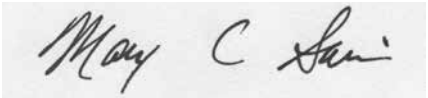
Mary Savin

Welcome to the 17th issue of *Discovery*, the journal that provides a venue for undergraduate students to disseminate their accomplishments in research and creative projects. I have had the honor of being the faculty editor for seven years, and the manuscripts submitted this year were some of the best we have received in my time working with this publication.

Discovery is a journal that was created for students of the Dale Bumpers College of Agricultural, Food and Life Sciences to facilitate the opportunity to complete the scientific process and disseminate results to a wide-reaching audience. The journal has been a valued outlet for students to hone written communication skills that will be critical for success in their professional careers. In fact many of these authors are Honors students and are graduating and moving onto to professional or graduate school or employment. The faculty members of the *Discovery* Editorial Board and the Dale Bumpers College are very proud of the accomplishments of these student authors.

This issue of *Discovery* continues the long-standing tradition of exceptional articles from students representing programs from across the college. There are 11 papers written by undergraduate student authors representing 7 departments and the School of Human Environmental Sciences working with 10 mentors. Six University of Arkansas System faculty and 13 other students and professionals were co-authors. These undergraduate students were certainly part of indispensable teams!

I welcome everyone to take time to read and reflect upon these projects and contributions. Please join with me in wishing the best for all Bumpers College students as they complete their journey here at the University of Arkansas and embark on their careers to become leaders, innovators, policy makers, and entrepreneurs in their professional careers.



Mary Savin, *Discovery* Faculty Editor
Professor of Microbial Ecology, Department of Crop, Soil, and Environmental Sciences

Undergraduate Research Articles

Then and now: across ten years of Arkansas women in agriculture

Paige Acklie[*]*, Jennie Popp*[†]*, Donald Johnson*[§]*, and Tamara Walkingstick*[‡]

Abstract

The United States Agricultural Census show that between 2002 and 2012, the number of women farm operators in Arkansas grew 14% (from 19,856 to 22,637). These women operators have made up an increasingly larger percentage of all farm operators in the state (from almost 29% to nearly 33%). There is little published information regarding changes over time in the role of women in agriculture, their challenges, and factors important to their success. While some surveys of farm women have been conducted, these surveys are generally insufficient because data exist only for one point in time. This research uses the first, middle and last years of survey data collected across ten years (2005-2014) at Arkansas Women in Agriculture (ARWIA) conferences to compare women's perceptions regarding: 1) factors important to their choice of business activity, 2) challenges women face in their agriculture-related business, and 3) the decision-making roles they hold in that business. Results suggest that women in Arkansas agriculture engage in important decision-making on the farm. These women consistently identified across all three years, three attributes—applying talents and skills directly, being involved in the community and being excited about the work—as important factors in their decision to choose an agricultural career. They also identified two problems—keeping good employees and finding/affording a good lawyer—within the top five of the largest challenges faced. It is hoped that this set of baseline information can be useful not only to researchers and educators interested in addressing needs of local women but also in illustrating the continuing changes in women's roles and their needs, and thus the need for extended research over time to address these changes.

[*] Paige Acklie is a May 2016 honors program graduate with a major in Agribusiness in the Department of Agricultural Economics and Agribusiness.

[†] Jennie Popp, the faculty mentor, is a Professor in the Department of Agricultural Economics and Agribusiness and Interim Honors College Associate Dean.

[§] Donald Johnson is a Professor in the Department of Agricultural Education, Communications and Technology.

[‡] Tamara Walkingstick is an Associate Professor and Associate Center Director with the University of Arkansas System Division of Agriculture's Arkansas Forest Resources Center, Little Rock.

Meet the Student-Author

Paige Acklie

I am from Highland Village, Texas and graduated from Marcus High School in 2012. I graduated in May 2016 from the Dale Bumpers College of Agricultural, Food and Life Sciences with a degree in Agribusiness and a minor in Agricultural Communications. Active in the Agricultural Business Club, I served as Treasurer my sophomore year, and President during my junior and senior year. I have served as a college ambassador for Bumpers College, Philanthropy Assistant for Delta Delta Delta, and am a member of the AgriBusiness/Agricultural Economics Quiz Bowl Team. During the summer of 2015, I studied abroad in Mozambique.

After my sophomore year, I interned in marketing with Sager Creek Vegetable Company in Siloam Springs, Arkansas. In February 2015, I began interning at Tyson Foods and am pursuing a career in commodity purchasing with Tyson in Springdale, Arkansas.

I would like to thank Jennie Popp for serving as my mentor for this project and Donald Johnson and Tamara Walkingstick for serving on my committee. Additionally, I would like to thank the Arkansas Women In Agriculture Association for their help with the administration and collection of this data throughout the years.

Introduction

Women contribute greatly to agricultural and rural society because of the roles they play on and off the farm. In 1978, there were 104,134 women who were the primary operators on farms and by 2007 that number had increased nearly 300% to 306,209 (Pilgeram and Amos, 2015). According to the United States Department of Agriculture (USDA) 2012 Census, nationally there are 969,672 women operators of farms (USDA, 2015a). Of those women, 29.7% of them are principal operators (USDA, 2015a). Between 2002 and 2012, the number of women operators in Arkansas grew 14% from 19,430 to 22,228 (USDA, 2015a, 2015b). The Arkansas agricultural sector contributes to the creation of over 280,000 jobs and adds $20.1 billion in total value to the state economy (English et al., 2014); therefore, the activities of Arkansas' women in agriculture are very important to the overall state economy, and are why women's roles have gained significant attention among policymakers and researchers. However, little is known in Arkansas regarding if and how agricultural women's roles, challenges, and important job attributes have changed over time.

In 2005, the first Arkansas Women in Agriculture (ARWIA) conference was established by faculty within the University of Arkansas System Division of Agriculture. Now a nonprofit organization, ARWIA's mission is to 1) provide education programs, 2) provide network opportunities and 3) identify new ways to balance the demands of family, community and professional lives (ARWIA, 2016). Since 2005 there has been a statewide conference held each year with the exception of 2013, a year when regional meetings were facilitated throughout the central, eastern, western, and southern parts of the state.

Some surveys have been conducted both nationally and internationally to examine farm issues; however, these surveys are generally insufficient because data exist for only one point in time. The purpose of this research is to use survey data collected at three of the ARWIA conferences to examine women's perceptions regarding 1) their roles on the farm, 2) the successes and challenges they face, 3) how their roles have changed over time, and 4) how that change has influenced their family lives, agriculture, and the rural community.

Materials and Methods

This research used survey data collected during three of the nine ARWIA conferences held between 2005 and 2014. These surveys were developed following methods suggested by Salant and Dillman (1994) and Dillman et al. (2009). The questions were designed with two types of attendees in mind: women who owned farms, ranches, or agribusinesses (Owners), and all other women attendees, whether they were farm employees or operators work-

ing in supporting industries, retired, or students (Non-owners). In total, there were 430 number of attendees at these three conferences. All attendees were encouraged to complete the survey.

The surveys consisted of six parts: 1) type of agricultural activity, 2) role in their business, 3) decision-making and other responsibilities in the business, 4) factors that are important in their jobs, and 5) challenges women face at their jobs and 6) demographic information (such as age, income, hometown, etc). The format of the questions ranged from multiple choice, open ended, and Likert-Scale (SD = strongly disagree, D = disagree, N = neutral, A = agree, SA = strongly Agree).

All survey responses were double-entered into an Excel spreadsheet and checked for accuracy. Then all respondents across the years were broken into the Owner and Non-owner groups. Chi square and Fisher Exact tests for categorical responses were conducted to look for differences ($P < 0.10$) in responses between Owners and Non-owners.

Tests were conducted on questions regarding: 1) factors important to their choice of business activity, 2) challenges women face in their agriculture-related business, and 3) the decision-making roles they hold in that business.

The null hypotheses tested in this research for Owners and Non-owners were:

- There is no significant difference in the job attributes that are important or the challenges faced, over time; further there is no difference in the top five job attributes or top challenges over time.
- There is no significant difference in the level of decision-making or the types of decisions made over time.

Results and Discussion

For the years 2005, 2009 and 2014 there were a total of 234 usable surveys, representing responses from 54.4% of attendees in those years. These women were equally split between Owners (117) and Non-owners (117). The largest single age group was 45-54 at 27.39% while 42.99% were under 45 years of age, and the remaining 29.62% were greater than 65 years of age. Only 10.48% had never been married, but 80.79% were married at the time the survey was completed. The remainder were either divorced, separated, or widowed. In total, 30.57% had a four-year college degree and 22.27% had some post graduate work. Figure 1 shows the top six counties (gray) that are most represented by the survey respondents: Wash-

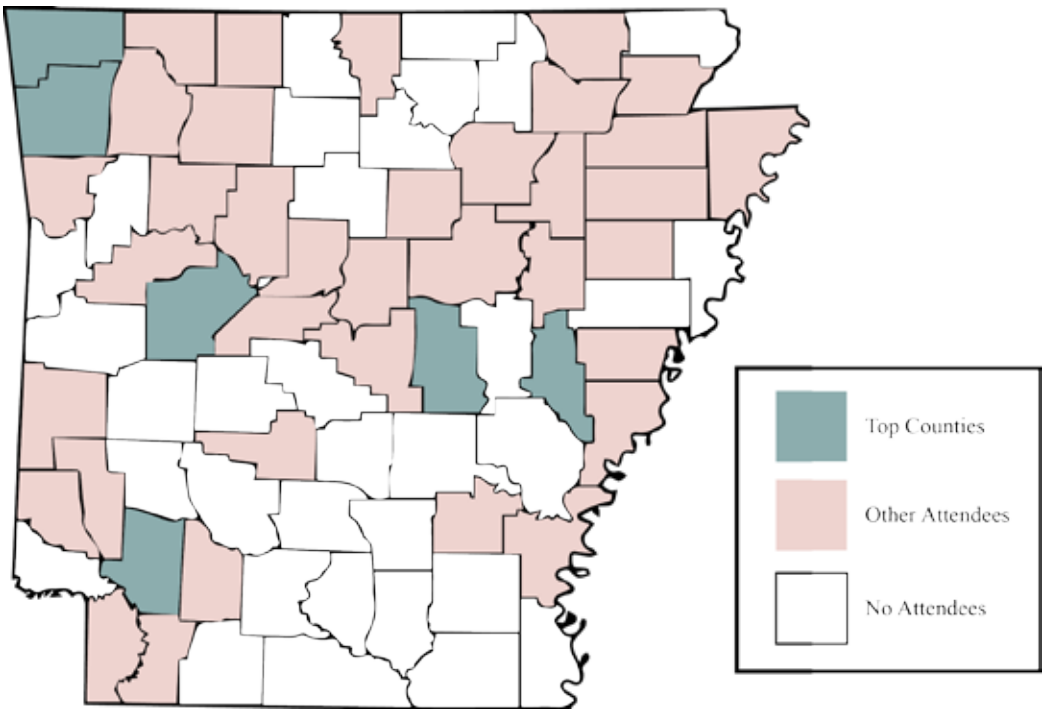


Fig. 1. 2005, 2009, and 2014 Arkansas Women in Agriculture conference attendees by county. The counties filled in with gray represent the top six counties in terms of survey respondents: Washington (11), Yell (11), Hempstead (9), Lonoke (8), Benton (7), and Monroe (7). The counties filled in with pink represent all other counties in attendance, while the counties not highlighted (white) did not have any attendees present at the 2005, 2009 and 2014 conferences.

Table 1. Level of agreement expressed by women Owners (%) that factors are important in their business.

	2005					2009					2014					
Factor	**SD**	**D**	**N**	**A**	**SA**	**SD**	**D**	**N**	**A**	**SA**	**SD**	**D**	**N**	**A**	**SA**	***P*-value**
I can apply my talents and skills directly	4.29	1.43	7.14	38.57	48.57	0	0	0	47.62	52.38	0	0	19.05	42.86	38.10	0.5197
I feel secure about my employment future	4.41	8.82	20.59	33.82	32.35	4.76	4.76	23.81	23.81	42.86	0	4.76	14.29	57.14	23.81	0.6317
I make the key decisions about the business	1.45	8.37	27.54	39.13	23.19	0	9.52	28.57	33.33	28.57	0	0	25.00	40.00	35.00	0.9098
I don't have to make key decisions about the business	27.69	26.15	27.69	16.92	1.54	35.00	25.00	20.00	15.00	5.00	33.33	38.10	14.29	9.52	4.76	0.7758
I am able to meet current financial needs	1.43	8.57	15.71	38.57	35.71	4.76	0	14.29	42.86	38.10	0	9.52	4.76	76.19	9.52	0.0549*
I can try new ways of doing things	0	1.45	11.59	52.17	34.78	4.76	0	4.76	61.90	28.57	0	0	14.29	66.67	19.05	0.5132
I can participate in environmental conservation practices (ag or non ag)	0	1.47	11.76	54.41	32.35	0	0	14.29	52.38	33.33	0	0	14.29	61.90	23.81	0.9643
I am excited about my work	1.43	0	12.86	42.86	42.86	0	0	4.76	38.10	57.14	0	0	14.29	38.10	47.62	0.8634
I have flexible work hours	0	5.71	11.43	47.14	35.71	4.76	14.29	4.76	42.86	33.33	0	0	28.57	47.62	23.81	0.1363
I can balance my work and free time	4.35	11.59	24.64	36.23	23.19	4.76	4.76	9.52	61.90	19.05	0	14.29	23.81	47.62	14.29	0.5741
I can be involved in my community	1.41	0	15.49	54.93	28.17	4.76	0	0	71.43	23.81	0	5.00	15.00	55.00	25.00	0.2342
I improve my standard of living	1.45	4.35	15.94	52.17	26.09	4.76	9.52	0	66.67	19.05	0	4.76	23.81	61.90	9.52	0.1684
I can pass the business onto a family member	0	4.29	21.43	38.57	35.71	0	4.76	23.81	33.33	38.10	0	4.76	4.76	47.62	42.86	0.6309
I provide jobs for my community	1.47	16.18	26.47	41.18	14.71	4.76	19.05	38.10	23.81	14.29	0	0	38.10	38.10	23.81	0.2771

Note: SD = Strongly Disagree, D = Disagree, N = Neutral, A = Agree, SA = Strongly Agree. *$P < 0.10$. **$P < 0.05$. ***$P < 0.01$.

Table 2. Level of agreement expressed by women Non-owners (%) that factors are important in their business.

	2005					2009					2014					
Factor	**SD**	**D**	**N**	**A**	**SA**	**SD**	**D**	**N**	**A**	**SA**	**SD**	**D**	**N**	**A**	**SA**	***P*-value**
I can apply my talents and skills directly	1.61	0	3.23	33.87	61.29	0	5.00	10.00	50.00	35.00	0	0	5.26	47.37	47.37	0.2110
I feel secure about my employment future	1.69	3.39	10.17	52.54	32.20	0	0	15.79	63.16	21.05	0	0	10.53	68.42	21.05	0.9025
I make the key decisions about the business	1.69	15.25	37.29	33.9	11.86	10.53	21.05	36.84	15.79	15.79	10.53	10.53	31.58	42.11	5.26	0.3925
I don't have to make key decisions about the business	8.33	23.33	35.00	21.67	11.67	25.00	20.00	15.00	25.00	15.00	26.32	31.58	26.32	10.53	5.26	0.2819
I am able to meet current financial needs	0	1.67	16.67	45.00	36.67	5.00	20.00	30.00	35.00	10.00	0	5.26	5.26	63.16	26.32	0.0061***
I can try new ways of doing things	0	0	16.67	50.00	33.33	5.00	5.00	25.00	40.00	25.00	0	0	15.79	68.42	15.79	0.1992
I can participate in environmental conservation practices (ag or non ag)	0	3.39	30.51	42.37	23.73	0	0	27.78	38.89	33.33	5.26	0	15.79	68.42	10.53	0.2595
I am excited about my work	1.61	1.61	8.06	41.94	46.77	5.26	5.26	5.26	57.89	26.32	0	0	5.26	63.16	31.58	0.4670
I have flexible work hours	1.59	7.94	9.52	50.79	30.16	5.26	10.53	15.79	47.37	21.05	0	5.26	10.53	57.89	26.32	0.9245
I can balance my work and free time	0	8.06	9.68	46.77	35.48	5.26	10.53	5.26	63.16	15.79	0	0	5.26	78.95	15.79	0.1337
I can be involved in my community	0	1.61	4.84	58.06	35.48	0	0	21.05	57.89	21.05	0	0	5.26	68.42	26.32	0.3573
I improve my standard of living	0	1.67	10.00	50.00	38.33	0	0	15.79	68.42	15.79	0	5.26	10.53	63.16	21.05	0.3585
I can pass the business onto a family member	5.08	10.17	44.07	20.34	20.34	10.53	10.53	36.84	36.84	5.23	10.53	21.05	31.58	31.58	5.26	0.3579
I provide jobs for my community	3.39	10.17	47.46	20.34	18.64	15.79	5.26	47.37	31.58	0	5.26	21.05	21.05	47.37	5.26	0.0210**

Note: SD = Strongly Disagree, D = Disagree, N = Neutral, A = Agree, SA = Strongly Agree. **P*< 0.10. ***P* < 0.05. ****P* < 0.01.

Table 3. Women Owner responses (%) that they encounter indicated challenges and problems in business activity.

	2005					2009					2014					
Factor	**SD**	**D**	**N**	**A**	**SA**	**SD**	**D**	**N**	**A**	**SA**	**SD**	**D**	**N**	**A**	**SA**	***P*-value**
Networking with others in similar activities	13.43	32.84	29.85	22.39	1.49	13.64	36.36	18.18	27.27	4.55	21.05	31.58	21.07	21.05	5.26	0.8700
Finding good information about production/agribusiness best management practices	8.96	37.31	2.09	28.36	4.48	18.18	40.91	13.64	18.18	9.09	10.00	20.00	45.00	20.00	5.00	0.2975
Finding information about government programs related to my type of business	7.35	35.29	14.71	29.41	13.24	9.09	40.91	13.64	22.73	13.64	5.00	35.00	25.00	30.00	5.00	0.9587
Qualifying for government programs related to my type of business	1.49	32.84	25.37	32.84	7.46	4.55	27.27	22.73	31.82	13.64	5.00	35.00	25.00	20.00	15.00	0.8080
Knowing where/how to market my products	10.14	30.43	30.43	20.29	8.70	9.09	27.27	22.73	22.73	18.18	10.00	45.00	20.00	20.00	5.00	0.8709
Keeping up with environmental regulations related to my activity	0	1.47	11.76	54.41	32.35	0	0	14.29	52.38	33.33	0	0	14.29	61.90	23.81	0.9643
Keeping financial records	10.00	28.57	21.43	27.14	12.86	4.55	27.27	18.18	27.27	22.73	15.00	40.00	15.00	25.00	5.00	0.8220
Finding/affording a good lawyer	2.94	20.59	29.41	30.88	16.18	4.55	9.09	36.36	31.82	18.18	5.00	20.00	30.00	30.00	15.00	0.9730
Keeping good employees	3.03	19.7	28.79	30.3	18.18	9.52	14.29	33.33	23.81	19.05	5.00	20.00	20.00	40.00	15.00	0.8947
Handling my cash flow	2.99	34.33	32.84	25.37	4.48	4.55	27.27	18.18	27.27	22.73	5.26	47.37	15.79	21.05	10.53	0.2645
Gaining access to credit	13.24	45.59	22.06	13.24	5.88	9.09	50.00	27.27	9.09	4.55	21.05	42.11	10.53	15.79	10.53	0.8832
Completing loan forms and other important paperwork	10.45	44.78	22.39	14.93	7.46	13.64	40.91	18.18	13.64	13.64	10.53	47.37	21.05	15.79	5.26	0.9935
Being respected in my industry as a female business person	5.88	32.35	19.12	22.06	20.59	13.64	40.91	22.73	13.64	9.09	10.53	52.63	15.79	15.79	5.26	0.5308

Note: SD = Strongly Disagree, D = Disagree, N = Neutral, A = Agree, SA = Strongly Agree. *$P < 0.10$. **$P < 0.05$. ***$P < 0.01$.

Table 4. Women Non-owner responses (%) that they encounter indicated challenges and problems in business activity.

	2005					2009					2014					
Factor	**SD**	**D**	**N**	**A**	**SA**	**SD**	**D**	**N**	**A**	**SA**	**SD**	**D**	**N**	**A**	**SA**	***P*-value**
Networking with others in similar activities	13.04	45.65	26.09	13.04	2.17	11.76	29.41	23.53	23.53	11.76	9.09	45.45	18.18	18.18	9.09	0.7367
Finding good information about production/agribusiness best management practices	15.91	31.82	38.64	9.09	4.55	17.65	17.65	41.18	11.76	11.76	8.33	66.67	16.67	0	8.33	0.2992
Finding information about government programs related to my type of business	6.82	36.36	40.91	13.64	2.27	11.76	23.53	41.18	11.76	11.76	9.09	45.45	18.18	18.18	9.09	0.5880
Qualifying for government programs related to my type of business	4.55	27.27	54.55	11.36	2.27	5.88	17.65	47.06	11.76	17.65	0	36.36	54.55	9.09	0	0.5942
Knowing where/how to market my products	9.09	34.09	34.09	22.73	0	11.76	17.65	35.29	23.53	11.76	9.09	63.64	18.18	9.09	0	0.2039
Keeping up with environmental regulations related to my activity	6.67	24.44	37.78	31.11	0	17.65	17.65	52.94	5.88	5.88	0	45.45	36.36	18.18	0	0.1284
Keeping financial records	18.18	25.00	29.55	22.73	4.55	11.76	29.41	41.18	11.76	5.88	9.09	36.36	36.36	9.09	9.09	0.9170
Finding/affording a good lawyer	6.82	11.36	54.55	18.18	9.09	0	5.88	52.94	29.41	11.76	0	18.18	54.55	18.18	9.09	0.9555
Keeping good employees	8.70	10.87	45.65	28.26	6.52	6.67	13.33	53.33	13.33	13.33	18.18	0	63.64	18.18	0	0.7362
Handling my cash flow	16.28	34.88	25.58	20.93	2.33	0	20	60	13.33	6.67	9.09	36.36	36.36	9.09	9.09	0.2645
Gaining access to credit	18.18	36.36	29.55	13.64	2.27	0	25	50	12.50	12.50	18.18	18.18	54.55	9.09	0	0.2602
Completing loan forms and other important paperwork	13.33	28.89	33.33	22.22	2.22	6.25	31.25	43.75	12.50	6.25	9.09	27.27	45.45	9.09	9.09	0.8718
Being respected in my industry as a female business person	8.70	21.74	32.61	28.26	8.70	12.50	12.50	25.00	37.50	12.50	0	20.00	30.00	50.00	0	0.9012

Note: SD = Strongly Disagree, D = Disagree, N = Neutral, A = Agree, SA = Strongly Agree. **P* < 0.10. ***P* < 0.05. ****P* < 0.01.

ington (11), Yell (11), Hempstead (9), Lonoke (8), Benton (7), and Monroe (7). Counties filled in with pink represent all other counties in attendance, while the counties not highlighted (white) did not have any attendees present at the 2005, 2009 and 2014 conferences.

All respondents were asked to indicate whether 14 job attributes were important to them in their operation/business (Table 1). When combining the agree and strongly agree responses, all attributes were important to at least 50% of Owner respondents each year except providing jobs to the community and not making key decisions. Two attributes, trying new ways of doing things and being excited about the work, were ranked within the top five most important attributes across all three years. Applying talents and being involved in the community ranked in the top five for two years. However, no statistical difference (at $P = 0.0.5$) was found across years for the attributes. Being able to meet current financial needs was cited more often as being important by 2014 than it had been in 2009. Non-owners shared many similarities with Owners, but in addition they highly valued being secure in their employment future and balancing work and free time (Table 2). The importance of providing jobs for the community ($P = 0.0210$) grew significantly over time for these women.

All respondents were asked if they faced challenges in their business related to 13 areas (Tables 3-4). It is important to note that in most cases less than half of the women agreed or strongly agreed that they faced any individual challenges. However, keeping up with environmental regulations was an exception as it was the top problem cited by over 85% of Owners each year. Finding/affording a good lawyer followed at 45% to 50%. No significant differences were found in any of the responses in this subset. Non-owners face many of the same problems as Owners, however a greater percentage of these women have challenges being respected in the industry, and while there is no significant difference across the three years, there was an increase in percentage between 2005 and 2009 from 37% to 50% and this percentage had not changed by 2014 from its 2009 level.

Respondents were asked if they share business making power, have sole decision-making power or have no power in decisions regarding their business/operations (Table 5). Over time, a larger numerical percentage of Owners have gained sole power in decision making (4.47% in 2005, 14.29% by 2014) while the Non-owners have no decision-making power (28.33% in 2005, 36.84% in 2014). However, there were no significant differences in the level of power across time for either group.

Respondents were asked who is involved in seven decision-making areas (Tables 6-8). As expected, these types of activities were generally more relevant to owners than to nonowners.

Table 5. Decision-making power in women's businesses.

	2005	2009	2014	*P*-value
Owners				
No decision-making power	4.17	0	0	
I share decision-making power	91.67	90.00	85.71	0.3599
I have sole power	4.17	10.00	14.29	
Non-owners				
No decision-making power	28.33	28.57	36.84	
I share decision-making power	66.67	57.14	57.89	0.6321
I have sole power	5.00	14.29	5.26	

Note: *$P < 0.10$. **$P < 0.05$. ***$P < 0.01$.

Table 6. Decision-making power in women's businesses.

	2005	2009	2014	*P*-value
Owners				
No decision-making power	4.17	0	0	
I share decision-making power	91.67	90.00	85.71	0.3599
I have sole power	4.17	10.00	14.29	
Non-owners				
No decision-making power	28.33	28.57	36.84	
I share decision-making power	66.67	57.14	57.89	0.6321
I have sole power	5.00	14.29	5.26	

Note: *$P < 0.10$. **$P < 0.05$. ***$P < 0.01$.

In one year, only one activity is an exception in which 12% or less of all women (owners and Non-owners combined) said that this activity was not relevant to their business and/or home roles at all. The percentage of Owners listing sole decision making increased between 2005 and 2009 for all areas except household expenditures. In nearly all cases, the percentage of women who had sole decision power in specific areas in 2014 reverted to 2005 levels. No significant differences were found. There were significant increases in the percentage of Non-owners who participated in decisions related to whether to buy equipment ($P = 0.0033$), where and when to sell products ($P = 0.0260$) and hiring workers ($P = 0.0812$).

Hypothesis Testing Results

Based on the results, we fail to reject the hypotheses for significant differences except in the cases of:

Job attributes

- Non-owners–providing jobs for the community and meeting financial needs

Types of decisions

- Non-owners–whether to buy major equipment; when/where to sell products and hiring workers

Numerically, numbers appeared very different in places between 2005 and 2009 as well as from 2009 to 2014. Research has suggested that the 2008-2009 world economic crisis had negative impacts on U.S. agriculture (Shane et al., 2009; Liefert and Shane, 2009). It led to a reduction in demand for U.S. exports and lowered commodity process compared to earlier years. As a result, in general, farm incomes fell and agricultural real estate lost some value. These impacts, felt mostly in 2009 and 2010, could help at least the numerical differences in 2009 compared to 2005 and 2014. Further statistical testing that includes analyses of other years between 2005 and 2014 is needed to truly evaluate whether statistical differences exist for the 2008-2009 years and other years.

Differences (though not statistically tested) were found in the ranking of many of the important job attributes and challenges when looking at Owners and Non-owners. Since Owners and Non-owners have different levels of control within the company, there are differences in the challenges they face. Generally, Owners are gaining more power within the company and are much more heavily involved in important business decisions; this can leave Non-owners with less opportunity for involvement. In addition, what is important to Non-owners will be very different from Owners because Non-owners reported the need to feel secure in their employment.

In many parts of the U.S., the number of women in agriculture and the number of women principal operators

Table 7. Owners (%) who are involved in decisions.

	2005				2009				2014				
	Self	Others	Self & Others	None	Self	Others	Self & Others	None	Self	Others	Self & Others	None	*P*-value
Whether to buy/sell/rent land or business property	8.70	5.80	81.16	4.35	17.39	8.70	73.90	0	16.67	0	77.78	5.56	0.6214
Whether to buy major equipment	5.88	10.29	80.88	2.94	17.39	21.74	56.52	4.35	16.67	11.11	61.11	11.11	0.1009
Types of farm/business practices used	10.45	17.91	71.64	0	12.50	12.50	70.83	4.17	11.11	5.56	77.78	5.56	0.4363
When/where to sell products	14.93	22.39	58.21	4.48	20.83	20.83	58.33	0	11.11	16.67	72.22	0	0.9271
Hiring workers	8.70	20.29	60.87	10.14	21.74	21.74	47.83	8.70	11.11	16.67	61.11	11.11	0.7779
Whether to borrow money	10.29	7.35	80.88	1.47	16.67	12.50	70.83	0	17.65	5.88	70.59	5.88	0.5913
How major household expenditures are made	34.78	2.90	59.42	2.90	26.09	0	73.91	0	33.33	0	66.67	0	0.9381

Note: $^{*}P < 0.10$. $^{**}P < 0.05$. $^{***}P < 0.01$.

is continuing to rise. Many organizations have formed in part to better understand the expanding roles of women in agriculture. This paper presents some of the findings of a research study aimed at understanding the structure of women in agriculture in Arkansas. While few significant differences existed over time, the majority of women surveyed played a sole or joint role in much of the business decision making. Additional analysis of the data is needed to help highlight the differences between Owners and Non-owners (if any) across individual years to help better understand if the roles of these two groups of women are diverging or coming together.

Acknowledgments

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Table 8. Non-owners (%) who are involved in decisions.

	2005				2009				2014				
	Self	Others	Self & Others	None	Self	Others	Self & Others	None	Self	Others	Self & Others	None	*P*-value
Whether to buy/sell/rent land or business property	4.35	21.74	69.57	4.35	22.22	11.11	66.67	0	14.29	14.29	42.86	28.57	0.2251
Whether to buy major equipment	4.35	52.17	30.43	13.04	20.00	0	70.00	10.00	14.29	0	42.86	42.86	0.0033***
Types of farm/business practices used	0	43.48	47.83	8.70	20.00	40.00	30.00	10.00	14.29	14.29	42.86	28.57	0.1774
When/where to sell products	0	50.00	40.91	9.09	20.00	40.00	20.00	20.00	28.57	0	57.14	14.29	0.0260**
Hiring workers	0	36.36	45.45	18.18	10.00	10.00	20.00	60.00	14.29	28.57	42.86	14.29	0.0812*
Whether to borrow money	8.70	26.09	60.87	4.35	20.00	10.00	50.00	20.00	28.57	14.29	42.86	14.29	0.4642
How major household expenditures are made	34.78	8.70	52.17	4.35	40.00	0	50.00	10.00	14.29	28.57	42.86	14.29	0.4887

Note: *$P < 0.10$. **$P < 0.05$. ***$P < 0.01$.

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Effect of timing of shade on growth, development, physiology, and fruiting of a primocane fruiting blackberry in a controlled environment

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Abstract

Primocane blackberry production in the upper south is limited by high temperatures during the bloom and early fruiting period, resulting in poor fruit set and poor fruit quality. Shade may have the potential to delay bloom and flowering to a more favorable season. A greenhouse study was established to evaluate the effects of shade on primocane blackberry growth, physiology, and fruiting. Single rooted plants of 'Prime-Ark® 45' were planted in 12-liter pots and grown in a greenhouse at the University of Arkansas System Division of Agriculture, Agriculture Research and Extension Center, Fayetteville, Arkansas. At approximately 0.25 m in height, one of the four following treatments was imposed with eleven single plant replications: 1) an untreated control (CK), 2) unshaded for 29 days then shaded for 30 days (US), 3) shaded for 29 days then shaded for 30 days (SS), and 4) shaded for 29 days and unshaded for 30 days (SU). Plants in the SU treatment were significantly taller than the SS and CK. Dry weight of leaves was consistent for all treatments except for SS which was significantly lower than the others. The CK bloomed first followed by US and SS. The last to bloom was the SU, 26 days after the CK. In conclusion, there was a delay of 'Prime-Ark 45' flower formation when 50% shade cloth was implemented and removed in the SU treatment. Further research needs to be completed to find the optimal intensity and timing of shade implementation that will improve fruit set in the southern region.

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Meet the Student-Author

Olivia Caillouet

I am from Little Rock, Arkansas, and graduated with honors from Little Rock Central High in 2012. I will graduate in December 2016 with a degree in Horticulture and minor in Sustainability. I was awarded 1st place in the southern regional American Society for Horticultural Science (ASHS) undergraduate oral paper competition February 2016 and received 3rd place in the poster competition at the National ASHS conference August 2015. Furthermore, I was awarded 2nd place in the Arkansas Academy of Science (AAS) oral undergraduate competition and received 3rd place in the Honors College Student Board Poster Competition in 2016. I am a State Undergraduate Research Fellowship (SURF) grant recipient for work in Fayetteville, Arkansas and Mozambique, Africa. During my time at the University of Arkansas I have served as the Horticulture Club treasurer and vice president; worked as the Bentonville Farmer's Market Assistant Manager; completed an internship on a certified organic citrus farm in Big Sur, California; and was a summer intern at a farm in Adjuntas, Puerto Rico. I plan to pursue graduate school after graduation and then embark upon my career focused on food security at the local as well as global scale.

I would like to thank Jason McAfee for all his help and guidance throughout this research process. Curt Rom was instrumental in this journey and his advice and support is appreciated. I would also like to thank my Honors Thesis committee members, Curt Rom, John Clark, Elena Garcia, and Lawton Nalley for the time and energy provided to make this process enjoyable and fulfilling. Lastly, thank you to my team Luke Freeman, Spencer Fiser, Julia Stover, and Heather Friedrich.

Introduction

Blackberry production in Arkansas, the region and the United States is increasing. Rodriguez et al. (2012) showed that the cultivated acreage of blackberry production in Arkansas increased 277% between the years of 1997 and 2007. The introduction of the autumn-bearing primocane-fruiting blackberry cultivars began with the release of 'Prime-Jan®' and 'Prime-Jim®' in 2004 by the University of Arkansas System Division of Agriculture (Clark et al., 2005). This unique type of blackberry fruits on current-season canes (primocanes) compared to traditional summer-fruiting blackberries which bear on second-season canes (floricanes) (Clark et al., 2005).

The new autumn-bearing, primocane fruiting blackberries expand the market season for the fruit. However, studies have shown that fruiting during hot seasons results in poor pollination, fruit set, and fruit quality. Stanton et al. (2007) tested three levels of temperature on primocane blackberry cultivars in growth chambers and it was found that increasing temperatures were directly correlated with lower percent of flowers and fruits. Primocane fruiting blackberries flower in Arkansas and the upper mid-South during July and August, traditionally the hottest months of the year. These new genotypes have not been found to be well adapted to Arkansas conditions.

The light environment can have an effect on flower formation and fruiting in rosaceae crops (Marini and Sowers, 1990). Based upon preliminary field experiments and observations (Curt Rom, pers. comm.), it was hypothesized that shade could delay flowering in primocane-fruiting blackberries. Based upon previous work, light saturation of blackberries occurred at 750-900 umoles/m^2/s^1 light flux which is approximately equivalent to 50% full sun on an average Arkansas day. Shade treatments would generally have allowed at or near light saturation allowing achievement of near maximum average photosynthesis rates (Curt Rom, pers. comm.). It is well studied that light is the driving energy source for photosynthesis which influences the rate of growth as well as development of plant organs (Janick, 1986). However, Janick (1986) states that when a plant reaches maturity, it is capable of flowering, but will not make the transition from a vegetative stem primordia into floral primordia unless the environment exposed to at the time of maturity is conducive.

A study on blackberries in a greenhouse tested a full sun control, 20%, 50%, and 70% irradiance to full sun (Gal-

lagher et al., 2014). Gallagher et al. (2014) reported the flower and fruit period was more concentrated when 70%-100% irradiance to full sun was implemented during initiation, meaning lower light levels may result in delayed flower differentiation and or incomplete development.

Rotundo et al. (1998) found that 40% shade reduction cloth extended the fruiting period 25 days for eight-year-old plantings of 'Black Satin' floricane blackberries and 28 days for 'Smoothstem' blackberries compared to the unshaded control in the Basilicata region of southern Italy at an altitude of approximately 630 m. Furthermore when shade was implemented in late July 1996 until late October, these two blackberry cultivars had an increased cumulative fruit production the following year, 1997, by 9% and 12%, respectively, compared to control (Rotundo et al., 1998). Through increasing or decreasing levels of light it is thought that the development of flower formation during the first three vegetative states: induction, initiation, and differentiation may be manipulated to shift primocane blackberry flower development.

There have been very few studies on the effects of shade on blackberries and no studies on the effects of shade on primocane blackberries were identified. Despite little research, there is reason showing adaptions to shading by blackberries. In a previous study, Rotundo et al. (1998) reports that two blackberry cultivars responded to reduced lighting under 40% shade netting through increased levels of chlorophyll production. Rates of photosynthesis, transpiration and stomatal conductance were also lower for shaded blackberry leaves (Rotundo et al., 1998). Makus (2010) states that two light-level treatments, 0% control and 40% shade, were implemented on blackberries 20 May 2008 and plants grown under shade had significantly higher cumulative yields compared to all other treatments. When 'Prime-Ark® 45' was released, it was reported that the first bloom date at the University of Arkansas System Division of Agriculture's Fruit Research Station, in Clarksville, Arkansas was 30 June and first ripe fruit was 8 Aug. which was the latest of the primocane cultivars tested (Clark and Perkins-Veazie, 2011). The date of shading for this experiment was chosen based on previous research so that light conditions would be altered during the vegetative stage of development.

Research in a controlled environment reduces variability and externalities that influence plant growth and development and therefore can isolate treatment effects.

Fig. 1. An illustration of the shade-unshaded treated plants of 'Prime-Ark® 45' day 36, 6 days after removing the initial shade treatment, while grown in a greenhouse, Fayetteville, Arkansas, 2014.

This has the potential to provide isolated treatment effects of various levels of shade on primocane-fruiting physiology with an emphasis on flower and fruit development. The objective of this study was to determine the effects of changing light environments on the growth and development of primocane fruiting blackberries. If these effects were observed, the flowering and fruiting period could be shifted to a more favorable season for fruit set and quality.

Materials and Methods

A greenhouse experiment was designed to complement a field experiment (Caillouet, et al., 2016) that evaluated the effects of various shade treatments on primocane-fruiting blackberries. The greenhouse is located at the University of Arkansas System Division of Agriculture's Arkansas Agricultural Research and Extension Center (AAREC), Fayetteville Arkansas (Latitude: 36º" N; Longitude: 94º" W). Experimental plants were grown in a double-layer, 6-mm polyethylene covered climate controlled greenhouse that is 12. 5 m (L) × 9 m (W) × 3 m (H) and has a north-south orientation. Greenhouse temperatures were controlled by a thermostatically controlled pad-and-fan cooling system during the summer with a 25/35 °C day/night temperature set point.

Plant Material and Management

Sixty bare-root dormant cuttings of 'Prime-Ark 45' were purchased from Berry and Plant Company (Plymouth, Indiana) and planted in 12-L pots using certified organic peatmoss and perlite based growing media (Sunshine® Natural and Organic Mix (Sungro Products) in early April, 2014.

When plants were approximately 0.25 m in height, 44 plants for the experiment were selected for uniformity of growth. During the study period, canes were pruned of axillary lateral bud break and trained to bamboo stakes. Every week suckers (adventitious shoots that arise from the base of the plant) were removed. When canes reached heights of approximately 1.5 m, the bamboo stakes were doubled to increase structural support for potted plants (Fig. 1). Blackberry plants were watered as needed. Potted plants were placed on wire benching systems and the height of the wire benches was lowered throughout the experiment as the plant's height increased.

Osmocote® fertilizer was applied in amounts of 15 g to each potted plant then lightly watered throughout the experiment. In addition, one application of insecticide (Imidacloprid) (Marathon®) was applied at a rate of 0.26 g/L until plants dripped on 28 July 2014 to control armyworms (*Spodoptera exempta*).

Treatments

After selection (described above), on 4 June 2014, selected plants were randomly assigned one of four treatments: 1) an untreated control (CK), 2) unshaded for 29 days then shaded for 30 days (US), 3) shaded for 29 days then shaded for 30 days (SS), and 4) shaded for 29 days and unshaded for 30 days (SU) (Fig. 2). Plants grew for 29 days at which time shade treatments were changed. Shade cloth was either added or removed 2 July 2014 to treatment 2) US, now shaded and treatment 4) SU, now unshaded for an additional 30 days with these treatments. After a 59-day period of treatments, all shade structures were removed and the plants were allowed to grow, flower, and fruit for an additional 30 days (Fig. 2).

Fig. 2. An illustration of all treated plants of 'Prime-Ark® 45' day 59, when all shade was removed, while grown in a greenhouse, Fayetteville, Arkansas, 2014.

Shade was provided by 50% shade neutral density cloth covering metal frame structures over the greenhouse benches. There were 11 single plant replicates of each treatment. Plants were placed with a single treatment per bench, and plants randomized within the bench surface. There was not a block design to this experiment due to limited greenhouse space.

Measurements

Starting the same week as treatments, measurements were taken. Weekly measurements of cane diameter (6 cm above the soil line), cane height (cm), estimated chlorophyll content (Minolta® SPAD) on the 4th or 5th leaf from the terminal cane tip and gas exchange (CIRAS-3® portable gas exchange monitor equipped with a Parkinson® leaf chamber) were taken once weekly over a period of 13 weeks. For chlorophyll estimates and gas exchange, the center most leaflet of the pentafoliate, four to five nodes below the terminal cane tip of each potted plant was used.

Leaf gas exchange was measured on a 6.25 cm^2 area of leaf. Cuvette-chamber conditions were set for incoming [CO_2] of 385 ppm, cuvette temperature of 28 °C, and inflow air relative humidity (RH) of 50%. Saturating light conditions of 1200 µmol/m^2/s^1 were provided with the PP Systems® PLC3 Universal LED Light head attached to the cuvette chamber. Gas exchange was measured after apparent steady-state conditions after 120-180 s.

The individual first date of replicated flower formation was recorded for each treatment and was not analyzed statistically. At the end of the 89-day study period, the final height (cm), cane diameter 6 cm above the soil line (mm), and number of flower buds, flowers, and fruits were recorded. Plants were destructively harvested. The total weight of buds (g), flowers (g), and fruits (g) was measured. The total leaf area (cm^2) and total number of leaves for each potted plant were recorded. After the fresh plant data were collected, the canes, stems, leaves, and reproductive organs were placed in paper bags within a dryer for 336 hours at 70 °C and weighted (g of dwt). Dry weight of leaves, stems, and roots was recorded to equal the total dry weight of plant biomass.

A completely randomized design was used for analysis. Data were analyzed with Proc GLM procedure in SAS statistical software (SAS v. 9.3, SAS Institute Inc., Cary, N.C.) and mean separation was calculated by least significant difference (LSD) (α = 0.05).

Results and Discussion

Plants in the SU treatment were the tallest compared to other treatments (Table 1, Fig. 3). The other treatments all had similar heights until shade was changed after 29 days for SU and US (Fig. 3). Treatments US and SU were greater than the CK, however SS was not different from CK or US (Table 1). The results for cane diameter were similar to cane height; SU had greatest stem diameter and SS was significantly thinner than SU while the control and US shoots were intermediate in diameter (Table 1).

The shade treatments affected plant biomass. The CK and SU treatments resulted in the greatest total plant bio-

Table 1. Final growth and harvest measurements of treated plants of 'Prime-Ark® 45' day 89, while grown in a greenhouse, Fayetteville, Arkansas, 2014.

	Shoots			Leaves		Total Fruit structures	% Open Flowers	% Closed Flowers	Total Fruit	Dry Weights			
Treatment	Height (cm)	Diameter (mm)	(No.)	Area (cm^2)	Avg. Size (cm^2)	(No.)	(No.)	(No.)	(No.)	Shoots (g)	Leaves (g)	Roots (g)	Total (g)
Control	197c†	9ab	41	5955	146	299	98	2	206	37a	53a	106a	196a
Unshaded/ Shaded	227ab	9ab	44	7917	189	250	95	5	72	35ab	51a	75b	162ab
Shaded/ Shaded	204bc	8b	42	7166	183	159	75	25	65	26b	34b	64b	124b
Shaded/ Unshaded	251a	10a	49	7077	145	143	74	26	37	42a	52a	94ab	188a
Prob > F	0.0025	0.05	ns	ns	ns	ns	ns	ns	ns	0.009	0.0017	0.0347	0.0185

† Mean separation followed by different letters are significantly different, α = 0.05. n = 11.
ns = there are no statistical differences among treatments.

mass, with no significant difference in the US but significantly less biomass in the SS treatment (Table 1). Plants shaded had reduced plant growth and development, especially dry weights. Although there were differences for height, cane diameter, and dry weights (shoots, leaves, roots, and total dry weight), there were no significant differences for other growth variables.

Leaf dry weight was similar for all treatments except for SS which was significantly less than the other treatments (Table 1). The results from this experiment agree with previous findings made by Marini and Sowers (1990) with another Rosacea species, peaches, in which specific leaf weight was found to decline with increased levels of shade.

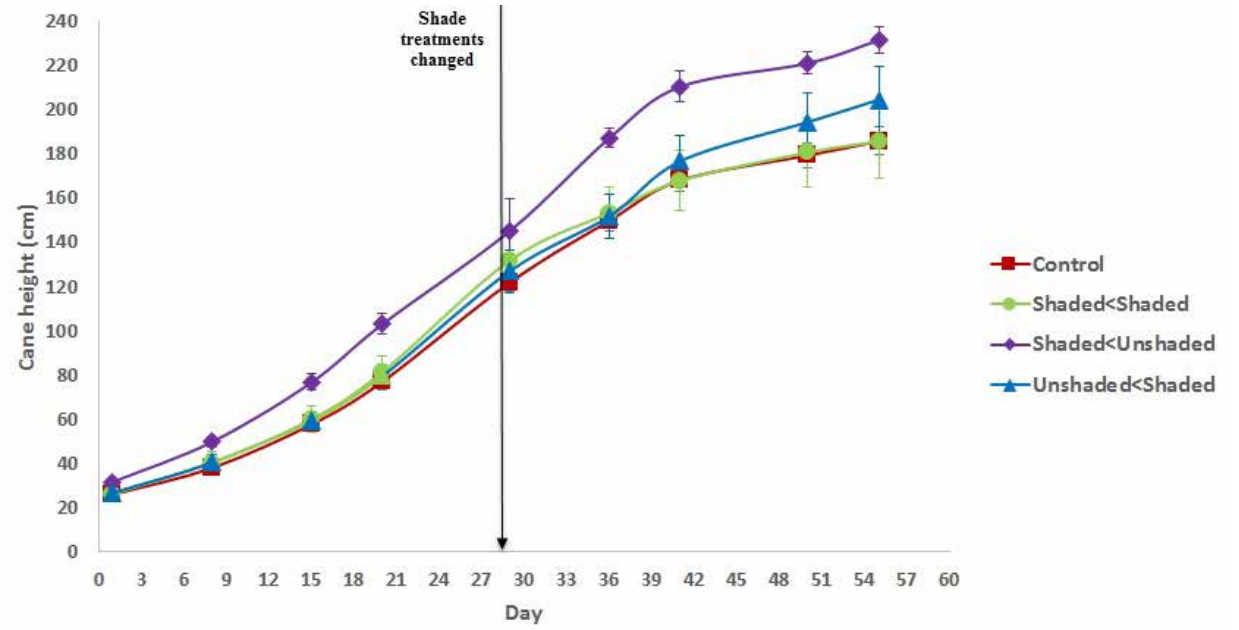

Fig. 3. Cane height (cm) of treated plants of 'Prime-Ark® 45', while grown in a greenhouse, Fayetteville, Arkansas, 2014. The vertical bars on the graph represent the +/- standard deviation in the data set. Standard deviation takes variances into consideration while increasing the statistical confidence of the results. The bar represents when shade treatments were changed. n = 11.

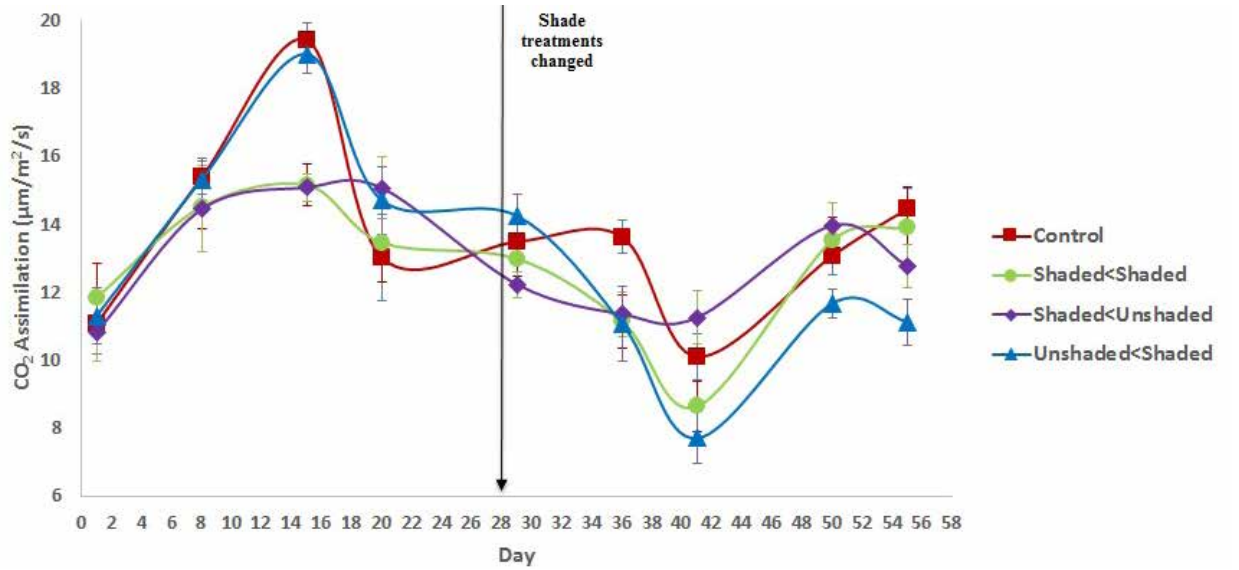

Fig. 4. CO_2 assimilation (A) of treated plants of 'Prime-Ark® 45', while grown in a greenhouse, Fayetteville, Arkansas, 2014. The bar represents when shade treatments were changed. n = 11.

Treatments CK and US, had the highest rates of CO_2 assimilation (A) at the start of the experiment and were different from SS and SU which were the least (Fig. 4). Plants adapted to the alteration in light conditions when shade treatments were changed as observed by the maintenance of similar A patterns within a treatment. The SS treatment adjusted to shading and was greater than US; all treatments were different from US at the conclusion of A data collection (Fig. 4).

After shade treatments were changed day 29 of the experiment, the estimated chlorophyll (CHL) content (SPAD) was greatest for CK and SU; while SS and US were the same and less than CK and SU (Fig. 5). At the end of the experiment when the final SPAD measurements were taken, SS and CK were the same and resulted in the highest SPAD values compared to other treatments; while SU and US plants were the same and had the least estimated CHL content (Fig. 5). This supports previous research findings that plants may adapt to continuous shade such as the SS treatment plants, which increased levels of estimated CHL content compared to other treatments and resulted in the same amounts as the CK (Fig. 5).

Flowers were distinguished depending on if they were opened flowers with petals or fruit compared to unopened flowers. The unopened flowers, opened flowers, and fruits were summed for total potential fruiting units (Table 1). The number of flower buds, flowers, and individual fruits did not vary significantly among treatments (Table 1).

For the first date of individual flower appearance, shading in the SU treatment resulted in a delay of flower and fruit set. The CK plants bloomed first 2 July followed by US on 17 July and SS on 27 July (Table 2). The last to bloom was the SU, 26 days after the CK on 28 July (Table 2). Given the research presented by Clark and Perkins-Veazie (2011) where fruit was formed 39 days after first flower, these findings are significant because fruit could be shifted to 5 Sept. compared to the CK which would fruit approximately 10 Aug. This shift of bloom time could be long enough to avoid heat stress that has been stated to be the challenge with primocane cultivars fruiting in Arkansas late July and August (Clark, 2008).

Results from the controlled environment greenhouse experiment support the original hypothesis that shading primocane fruiting potted plants does influence plant physiology, growth, and development. This experiment met the objective to gain further insight into effects of 50% shade cloth on primocane fruiting blackberries. Further research is needed, with different levels of shade as well as the translation of information to field production systems in the southern region to determine if shade can be used effectively and economically to shift the flowering period of primocane blackberries without significant negative effects on growth.

Acknowledgements

This research was made possible by a grant funded by Southern Sustainable Agriculture Research and Education (SSARE; LS12-250) and an additional S-SARE Young Scholar Enhancement apprenticeship grant. Addi-

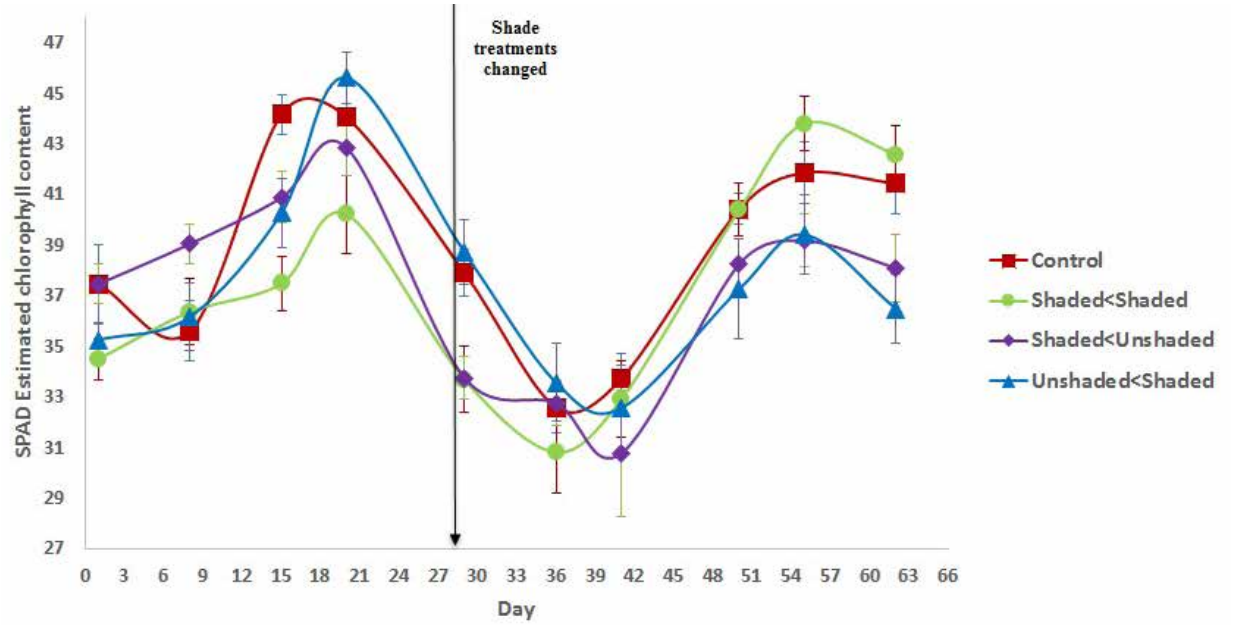

Fig. 5. Estimated chlorophyll content measured with Minolta SPAD-502 Plus® monitor of treated plants of 'Prime-Ark® 45', while grown in a greenhouse, Fayetteville, Arkansas, 2014. The bar represents when shade treatments were changed. n = 11.

tional grant funding was provided by the Dale Bumpers College of Agricultural, Food and Life Sciences and Honors College of the University of Arkansas undergraduate research grant programs. Support also provided by the University of Arkansas System Division of Agriculture. This project was part of an undergraduate Honors Thesis. Travel to present research was partially supported by the University of Arkansas Department of Horticulture Mitchener Undergraduate Scholarship Award.

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Table 2. Date of first flower blooming within a treatment group of 'Prime-Ark® 45' blackberry grown in pots as affected by four shade treatments while grown in a greenhouse, 2014, Fayetteville, Arkansas.

Treatment	Date
Control	2 July 2014
Unshaded, Shaded	17 July 2014
Shaded, Shaded	26 July 2014
Shaded, Unshaded	28 July 2014

There are no statistical differences in the table above.

The effects of shade on primocane fruiting blackberries in the field

Olivia C. Caillouet[*], *Curt C. Rom*[†], *Jason McAfee*[§], *Luke Freeman*[‡], *and Heather Friedrich*[#]

Abstract

Primocane fruiting blackberry production in Arkansas is limited by heat during the flowering and early fruiting season. Shade could be used to delay flowering and fruiting to more favorable growth period. This study was designed to test three levels of shade (0% [control], 30% and 50% shading) applied at three times during the growing season that examined the growth, development, physiology of flowering, and fruiting of 'Prime-Ark® 45' blackberries. The seven treatments were as follows: 1) an untreated control (CK), 2) early shade 30% (ES30), mid shade 30% (MS30), 4) late shade 30% (LS30), 5) early shade 50% (ES50), 6) mid shade 50% (MS50), and 7) late shade 50% (LS50). The 30% and 50% treatments were implemented 16 June (ES) and left on for 95 days, 1 July (MS) and left on for 80 days, and 15 July (LS) and left on for 66 days. All shade was removed 19 Sept. 2014. Foliar gas exchange using CIRAS®-3 portable gas exchange monitor and estimated chlorophyll content (Minolta SPAD®) were measured weekly. Beginning at maturity, fruit was harvested biweekly to determine fruit yields per plot. Plant growth was measured destructively at the end of the study period. The cumulative berry weight was greatest for LS50 and LS30 which was not different from the CK or MS50, while ES30, MS30, and ES50 berry weights were significantly less. The cumulative marketable weights were greatest for LS30 and CK, while ES30 and MS30 were less than the CK. Shade altered flower and fruit production, but was not found to result in higher fruit quantities compared to the control. Some ES treatments reduced cropping compared to LS treatments.

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Meet the Student-Author

Olivia Caillouet

I am from Little Rock, Arkansas, and graduated with honors from Little Rock Central High in 2012. I will graduate in December 2016 with a degree in Horticulture and minor in Sustainability. I was awarded 1st place in the southern regional American Society for Horticultural Science (ASHS) undergraduate oral paper competition February 2016 and received 3rd place in the poster competition at the National ASHS conference August 2015. Furthermore, I was awarded 2nd place in the Arkansas Academy of Science (AAS) oral undergraduate competition and received 3rd place in the Honors College Student Board Poster Competition in 2016. I am a State Undergraduate Research Fellowship (SURF) grant recipient for work in Fayetteville, Arkansas and Mozambique, Africa. During my time at the University of Arkansas I have served as the Horticulture Club treasurer and vice president; worked as the Bentonville Farmer's Market Assistant Manager; completed an internship on a certified organic citrus farm in Big Sur, California; and was a summer intern at a farm in Adjuntas, Puerto Rico. I plan to pursue graduate school after graduation and then embark upon my career focused on food security at the local as well as global scale.

I would like to thank Jason McAfee for all his help and guidance throughout this research process. Curt Rom was instrumental in this journey and his advice and support is appreciated. I would also like to thank my Honors Thesis committee members, Curt Rom, John Clark, Elena Garcia, and Lawton Nalley for the time and energy provided to make this process enjoyable and fulfilling. Lastly, thank you to my team Luke Freeman, Spencer Fiser, Julia Stover, and Heather Friedrich.

Introduction

When temperatures are above 29.4 °C, heat stress has been found to be detrimental to flower and fruit production of autumn bearing primocane blackberries (Stanton et al., 2007). This limits the production of the new cultivars of primocane blackberries for production in Arkansas which begin flowering in July or August during times of high temperatures. Observations and a preliminary study in 2013 indicated that shading may be used to delay and synchronize bloom to a cooler, more favorable environment in autumn-bearing primocane blackberries. A field study was conducted 2014 to evaluate the effects of various levels of shade applied at different times throughout the growing season on 'Prime-Ark® 45' blackberries in order to confirm previous observations.

Blackberry demand and production worldwide are increasing by advanced cultivars, and with high tunnel and field production systems (Strik et al., 2007). Small fruit crops, blackberries in particular, are economically viable and could serve as a sustainable income for farmers while supplying consumers in the southern region with local produce. Traditional blackberries are a biennial plant with the first year cane—the primocane—arising from a perennial root system, remaining vegetative. After a winter dormant period, the second-year cane—the floricane— flowers in spring, fruits, and dies. A new genotype of an autumn-bearing fall harvested primocane fruiting blackberries have been developed at the University of Arkansas System Division of Agriculture. Superior cultivars of the primocane fruiting autumn-producing blackberries are being released and being grown. This has significantly expanded the blackberry production and market season.

Although very productive in cooler climates, these new genotypes have limited adaptability in Arkansas due to high temperatures during the flowering and fruit set period of July and August. It has been suggested that shade cloth could reduce fruit temperatures while also increasing fruit size and the amount of marketable berries with crop season extension (Makus, 2010). Therefore, there are two proposed methods for improving fruit of primocane cultivars: one method is to shade fruit, while a second is implement shade during flower production to shift fruit to a time where heat is avoided. The light treatments during flower formation were not meant for fruit temperature reduction in this study. It has been thought that shade may delay flowering of primocane-

bearing cultivars to a more favorable season, although the research is scarce. The purpose of this study was to use light as a means of shifting the flower and fruit fruiting sequence of primocane blackberries to avoid heat.

Based upon previous work, light saturation of blackberries occurred at 750-900 umoles/m^2/s light flux which is approximately equivalent to 50% full sun on an average Arkansas day. Shade treatments would generally have allowed at or near light saturation allowing achievement of near maximum average photosynthesis rates (Curt Rom, pers. comm.). It is well studied that light is the driving energy source for photosynthesis which influences the rate of growth as well as development of plant organs (Janick, 1986). Plant organs such as stems, leaves, and flowers reach a genetically programmed minimal age of development, which varies by species and determines when the plant is capable of flower formation (Durner, 2013). However, Janick (1986) states that when a plant reaches maturity, it is capable of flowering, but will not make the transition from a vegetative stem primordia into floral primordia unless the environment it is exposed to at the time of maturity is conducive.

A study on apple trees, another rosacea species implemented three treatments: a nonshaded control, continuous 80% shade, and intermittent shade that provided both full sun and full shade (Barden, 1977). The experiment by Barden (1977) found that plant growth was dependent upon accumulated photosynthetically active radiation rather than the level of light provided. A study on blackberries in a greenhouse tested a full sun control, 20%, 50%, and 70% irradiance to full sun (Gallagher et al., 2014). Gallagher et al. (2014) reported the flower and fruit period were more concentrated when 70%-100% irradiance to full sun was implemented during initiation, meaning lower light levels may result in delayed flower differentiation and or incomplete development. It is proposed in this experiment that the use of 30% and 50% shade isolated the light intensity factor and would not reduce the photosynthetically active radiation required for growth, but delay vegetative bud development.

Flower bud initiation of several primocane fruit blackberry cultivars under field conditions was statistically different when number of nodes reached 25 between 14 and 28 May 1997 (Lopez-Medina et al., 1999). This research was the first of its kind and provided the foundation to further understand primocane blackberry flower initiation development under nonshaded conditions, which may be used to manipulate flower development in the future (Lopez-Medina et al., 1999). This previous research gave insight for determining when shade treatments (ES, MS, and LS) would be implemented in the field for this experiment. Rotundo et al. (1998) found that 40% shade reduction cloth extended the fruiting period 25 days for eight-year-old plantings of 'Black Satin' floricane blackberries and 28 days for 'Smoothstem' blackberries compared to the unshaded control in the Basilicata region of southern Italy at an altitude of approximately 630 m. Furthermore when shade was implemented in late July 1996 until late October, these two blackberry cultivars had an increased cumulative fruit production the following year, 1997, by 9% and 12%, respectively, compared to the control (Rotundo et al., 1998). Through increasing or decreasing levels of light, it is thought that the development of flower formation during the first three vegetative states—induction, initiation, and differentiation—may be manipulated to shift primocane blackberry flower development. The objective of this study was to determine if various levels of shade (30% and 50%) used at different times of the pre-flowering season (ES, MS, and LS) could alter the flowering and fruiting season of a new genotype of autumn-producing primocane fruiting blackberries in Arkansas. The hypothesis was that shade applied pre-flowering would delay bloom and harvest.

Materials and Methods

Location

The field experiment is located in the organic block of the University of Arkansas System Division of Agriculture's Agricultural Research and Extension Center in Fayetteville, Arkansas (Latitude: 36°6'8" N; Longitude: 94°10' 17" W). The field was managed using National Organic Production (NOP, 2014) standards which enforce regulations on organic food production in the United States.

Plant Materials and Experimental Design

An experimental planting 'Prime-Ark® 45' primocane fruiting blackberry was established in spring 2011; and in 2013, a study evaluated cultural practices related to primocane production. 'Prime-Ark® 45 plants were obtained from Boston Mountain Nurseries. Plants were grown in the field with Captina (Fine-silty, siliceous, active, mesic Typic Fragiudult) silt loam soil. Plants were planted in 6 rows, at 30.5-cm intervals within the row with 2.7 m between rows.

Plot Management

Canes were cut back to the crown each winter after harvest and new primocanes which emerged approximately 1 April were thinned to approximately five canes per crown in the spring with others being removed by pruning. Canes were tipped (removing the growing tip) one time on 6 June when canes were approximately 1 m in height to encourage lateral bud break. The field plot was irrigated as needed according to Irrometers®. The irrigation was inline drip tube with 30.5-cm spacing and a

flow rate of 1.9 L/hour. Plants were fertilized every spring using Bradfield Organics® Luscious Lawns Mix (3-1-5) which was applied in banded rows. For seasonal pest control, plants were sprayed for spotted winged drosophyla using (Spinosad, Naturalyte® Insect Control) at a rate 0.01 L/0.40 ha.

The study was designed to test three levels of shade (0% [control], 30%, and 50% shading) applied for 95-, 80-, and 66-day periods at three different times during the summer growing cycle (Fig. 1). The field study had seven treatments with various levels of shade and differing dates of treatment implementation as follows: 1) an untreated control (CK), 2) early shade 30% (ES30), 3) mid shade 30% (MS30), 4) late shade 30% (LS30), 5) early shade 50% (ES50), 6) mid shade 50% (MS50), and 7) late shade 50% (LS50). There were five replication plots for all treatments. The 30% and 50% treatments were implemented 16 June (ES), 1 July (MS), and 15 July (LS) during the 2014 summer season. Buffer plots were established between treatment plots to isolate treatments. Shade structures were placed over 1.8-m row sections. Size and dimension of shading structure were 1.5 m (L) × 1.2 m (W) × 2.1- 2.4 m (H). Any previously formed flowers at the onset of treatments were removed from canes under shade treatments when cloths were implemented on 16 June. This was done to ensure uniformity among treatment plots and provide accurate observations regarding effects on shade flower and fruiting formation. The experiment was designed in a 3 × 3 factorial of shade by time treatments plus an untreated control with five replicated plots of each treatment in a completely randomized design.

Research Variables and Data Collection

Two healthy, vigorous canes in each treatment plot were tagged as sub-samples. The primocanes were selected for uniformity, growth, and overall health, and as a representative sample of the plot.

Measurements began approximately 1.5 hrs after sunrise beginning at 7:30 AM (CDST) lasting until 12:00 PM or until all plots were recorded in a randomized order. The center most leaflet of the blackberry pentafoliate on a leaf located four to five nodes from the tip was used for each reading. Chlorophyll estimates were made with the Minolta model SPAD-502 Plus® monitor measured on the same leaf used for foliar gas exchange measurements.

Plots began to fruit 60 days after first shade treatment beginning on 18 Aug. Fruit was harvested from plots twice every seven-day period (Fig. 2). Days after treatment (DAT) is the number of days since ES was implemented on 16 June and is used to describe measurements as well as fruit harvest data. Towards the end of the study period, the ripe fruit was harvested once every seven-day period. The total berry weight (g) for each treatment was recorded (Fig. 2). The study harvest period lasted 50 days.

Fig. 1. Collecting CIRAS®-3 portable gas exchange monitor measurements after implementation of early shade (ES) and middle shade (MS) cloth of 'Prime-Ark® 45' blackberry as affected by seven shade treatments while grown in the field, at the University of Arkansas System Division of Agriculture's Arkansas Agricultural Research and Extension Center, Fayetteville, Arkansas, 2014.

The blackberries were sorted into marketable and unmarketable fractions based upon observed fruit quality and characteristics. Criteria for the marketable berries were firmness, size, without disease or mold and limited punctures to druplets. Once graded, the total weight of unmarketable berries and total weight of marketable berries for each plot was recorded. Then the weight of 25 randomly selected marketable berries for each plot was recorded and used to determine the average weight per marketable berry. Twice during the harvest collection of berries, 29 Aug., and 12 Sept., five randomly selected marketable berries were measured for the soluble solids content.

After the conclusion of fruit harvest on 19 Oct., the tagged canes were destructively harvested for growth measurements which included: cane diameter (6 cm above the soil line) (mm), cane shoot length (cm), number of nodes, number of lateral branches formed after pruning, number of flower clusters per cane, and the number of fruit clusters per cane.

Results and Discussion

The estimated chlorophyll content at 36 DAT of plants in the LS50 treatment was statistically greater than all other treatments except CK and LS30 (Table 1). At DAT 36, there were no treatments that had chlorophyll contents significantly different from the CK. However, at DAT 45, the CK, MS30, LS30, and LS50 had greater chlorophyll contents than MS50, while ES30 was not different from any other treatments. These data indicate that over the course of the experiment, there were only two days out of eight when statistical differences were measured for chlorophyll content among treatments (Table 1).

Fruits were harvested beginning at 60 days after the onset of the experimental treatments. There was no apparent difference in the dates of first harvest among the treatments. Plants in the LS30 and LS50 treatments produced greater cumulative yield berry weight than ES30, MS30, and ES50 treatments, while all treatments were not different from the CK (Fig. 3). The cumulative harvested berry

Fig. 2. Fruit harvests of 'Prime-Ark® 45' blackberry as affected by seven shade treatments while grown in the field, at the University of Arkansas System Division of Agriculture's Arkansas Agricultural Research and Extension Center, Fayetteville, Arkansas, 2014.

Table 1. Estimated leaf chlorophyll content measured by Minolta SPAD-502 Plus® monitor of 'Prime-Ark® 45' blackberry as affected by seven shade treatments while grown in the field, at the University of Arkansas System Division of Agriculture's Arkansas Agricultural Research and Extension Center, Fayetteville, Arkansas, 2014.

	SPAD Estimated leaf chlorophyll content							
Treatment	**DAT‡ 10**	**DAT 15**	**DAT 27**	**DAT 36**	**DAT 45**	**DAT 57**	**DAT 64**	**DAT 69**
Control	38	35	37	40.7abc†	42.3a	44	47	47
Early shade 30%	40	31	36	36bc	38.9abc	43	45	47
Middle shade 30%	38	35	36	39bc	41.9ab	44	48	49
Late shade 30%	41	36	39	41ab	41.3ab	42	46	48
Early shade 50%	37	28	37	35c	38bc	44	44	44
Middle shade 50%	35	37	38	35c	36c	43	45	45
Late shade 50%	41	35	41	45a	42.4a	44	47	49
Prob > F	0.5	0.1	0.3	0.01	0.03	1	0.5	0.1

† Mean comparisons among treatments were calculated using SAS Proc GLM least significant difference. Means followed by a similar letter are not different. ($\alpha < 0.05$, n = 5).

‡ DAT = Days after treatment.

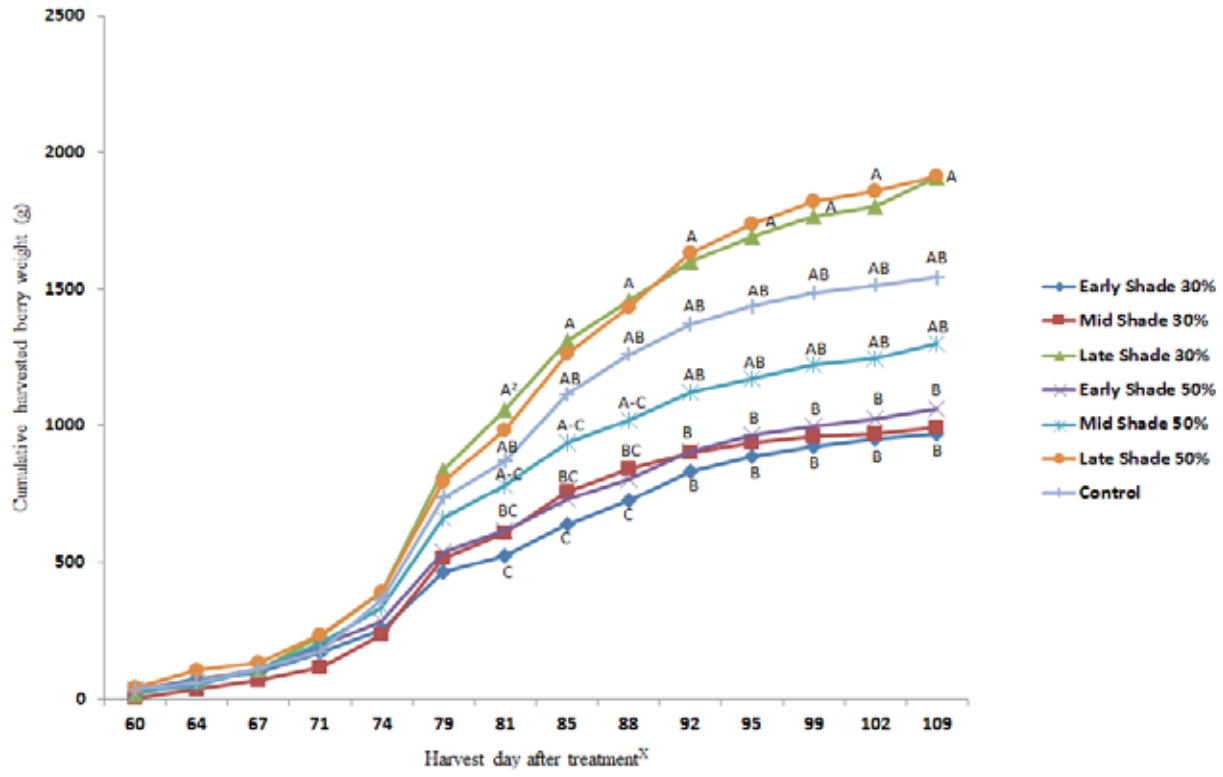

Fig. 3. Cumulative harvested berry weight (g) of 'Prime-Ark® 45' blackberry as affected by seven shade treatments while grown in the field at the University of Arkansas System Division of Agriculture's Arkansas Agricultural Research and Extension Center, Fayetteville, Arkansas, 2014. Mean comparisons among treatments were calculated using SAS Proc GLM LSD. Means followed by a similar letter are not different. ($\alpha < 0.05$, n = 5). DAT= Day after treatment.

weight which was greatest for LS30 and LS50 began to differentiate in harvest berry weight from ES30 starting 80 DAT and continued until the conclusion of the experiment, 110 DAT (Fig. 3). At approximately 95 DAT, both LS30- and LS50-treated plants had average yields above 1500 g per plot compared to ES30, MS30, and ES50 that had average berry yields less than 1000 g (Fig. 3).

After sorting fruit to segregate marketable and nonmarketable fruit, the mean cumulative marketable yields were 269% greater for LS30-treated plants compared to ES30-treated plants which were the least (Table 2). There were no statistical differences among treatments for soluble solids, cumulative unmarketable or culled berry weights (data not shown).

No significant difference for cane length, cane diameter, node number, internode length, number of lateral branches or number of fruit clusters was observed among treatments (data not shown). The short-term shade treatments were made after canes were tipped, setting their final height, and after lateral bud break had occurred. Therefore, shade did not affect gross growth in this experiment.

The hypothesis was that that shade would affect flower formation and subsequently fruit formation of primocane blackberries in the field. There was no effect on plant growth, and some shade treatments did reduce yield. Treatments ES30, MS30, and ES50 had less fruit than LS treatments. Gallagher et al. (2014) stated that flower and fruit were more concentrated when lower light levels were implemented during the flower initiation stage. Since previously formed flowers were removed prior to the ES treatments, it is possible that shade was not applied early enough during the vegetative stages of initiation. This could explain why there was no difference in plant growth, but yields were lower in some ES treatments. If that was the case, in the future shade should be applied 1 May as opposed to 16 June. Earlier shade could be coupled with season-extending high tunnel systems to protect fruit against freezing autumn weather that would end field production. This is the first research of its kind and more work needs to be completed to determine if shade is a possible management tool for delaying flower formation and cropping. The potential of shading in combination with high tunnels may provide an opportunity for primocane fruiting, autumn-bearing blackberries in Arkansas and the southern region of the United States.

Acknowledgments

This research was made possible by a grant funded by Southern Sustainable Agriculture Research and Education (SSARE; LS12-250) and an additional S-SARE Young Scholar Enhancement apprenticeship grant. Additional grant funding was provided by the Dale Bumpers College of Agricultural, Food and Life Sciences and Honors College of the University of Arkansas undergraduate research grant programs. Support also provided by the University of Arkansas System Division of Agriculture. This project was part of an undergraduate Honors Thesis. Travel to present research was partially supported by the University of Arkansas Department of Horticulture Michener Undergraduate Scholarship Award.

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Table 2. Cumulative marketable yield berry weight (g) of 'Prime-Ark® 45' blackberry as affected by seven shade treatments while grown in the field at the University of Arkansas System Division of Agriculture's Arkansas Agricultural Research and Extension Center, Fayetteville, Arkansas, 2014.

Treatment	Cumulative marketable yield (g)
Control	585ab[†]
Early Shade 30%	244d
Middle Shade 30%	355cd
Late Shade 30%	657a
Early Shade 50%	399b-d
Middle Shade 50%	473a-c
Late Shade 50%	579ab
Prob > F	0.006

[†] Mean comparisons among treatments were calculated using SAS Proc GLM least significant difference. Means followed by a similar letter are not different. ($\alpha < 0.05$, n = 5).

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Evaluation of protein source at breakfast on energy metabolism, metabolic health, and food intake: a pilot study

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Abstract

Over 30% of adults in the U.S. are obese. A primary contributor to obesity is an unhealthy diet related to imbalanced macronutrients. Diets higher in protein (PRO) rather than carbohydrate (CHO) are associated with increased energy expenditure (EE) and reduced food intake. The objective of this pilot study was to determine if protein source at breakfast influences EE in young men (n = 4; ages 18-35). Participants consumed three isocaloric (whey (WP), pea (PP), beef (BP); 275 kcal, 62% PRO, 23% CHO, 15% Fat) drinks in a randomized, crossover design study with a one-week washout period (time between the administration of each treatment to control for potential interactions). Each test day EE, appetite, and cravings were assessed at 0, 15, 30, 60, 120, 180, and 240 min following consumption. Data were analyzed using 2-way analysis of variance (ANOVA) for effects of protein source over time and one-way ANOVA for area under the curve (niAUC). Resting EE niAUC was 8% lower in BP vs PP and 5% lower vs WP. Thermic effect of feeding niAUC was 77% lower in BP vs WP; PP was 43% lower than WP. Carbohydrate oxidation was higher (31%) with PP compared to WP with no difference between BP and WP. Fat oxidation was 23% higher in WP vs BP and PP. The WP was most satiating. Participants had a higher craving for sweet foods following PP and a higher desire for snacks following BP. Food intake post-treatment was similar in calories and macronutrient distribution. Lack of significant difference among measurements suggests that protein source is not a predictor of postprandial EE, appetite response, or food intake.

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[‡] Jamie I. Baum, faculty mentor, is an assistant professor in the Department of Food Science.

Meet the Student-Author

Lauren Cambias

I was raised in Batesville, Arkansas, I graduated with honors from Batesville High School in the spring of 2012 and moved to Fayetteville to begin college at the University of Arkansas. I then graduated from the University of Arkansas in the spring of 2016 with a Bachelor of Science in Human Environmental Sciences, majoring in Human Nutrition and Hospitality Innovation. Throughout my four years of undergraduate study I was able to serve as Hall Senate Treasurer for Holcombe Hall; be an active member of Gamma Beta Phi honors society; take part in the Student Dietetics Association; serve as Secretary, Vice-President, and President of the Registered Student Organization United Campus Ministry; and have a breathtaking study abroad experience in Rome, Italy over the summer of 2014. I would like to thank Jamie Baum for being my honors thesis mentor, for taking time to explain the research process as we went, and for meeting and corresponding with me when I needed guidance. I would like to especially thank Brianna Neumann for teaching me metabolic cart skills and all of her contributions to the research. In addition, I would like to thank Stephanie Shouse, Charlayne Mitchell, and Enela Silva for their help in conducting this research. I am excited to say I will begin a combined Dietetic Internship and Master's Degree Program at the University of Texas Medical Branch in the fall of 2016.

Introduction

More than one-third of U.S. adults—78.6 million—are obese (Ogden et al., 2014). As consumers grow concerned for their health, nutrition researchers endeavor to provide evidence that supports obesity prevention, weight control, and weight loss. The consumption of plant-based proteins as substitutions for and alternatives to animal-based proteins have been recommended in recent years (Douglas et al., 2015).

Dietary protein may play an important role in opposing the obesity epidemic Americans currently face (CDC, 2014; Douglas et al., 2015; Millward et al., 2008; Veldhorst et al., 2008; Veldhorst et al., 2009). Protein in the diet may be beneficial for weight loss and weight maintenance due to protein's satiating properties. Feelings of satiety between meals greatly contribute to appetite and caloric intake throughout the day (Weigle et al., 2005). Proteins eaten at earlier meals (e.g., breakfast, lunch) may have an effect on the quantity of foods chosen for consumption at later meals, decreasing the amount consumed and preventing overeating (Anderson and Moore, 2004; Lang et al., 1998; Leidy et al., 2013; Weigle et al., 2005). In addition, several studies have found that fat intake, as well as protein and carbohydrate intake, was lower after consuming high protein meals (Latner and Schwartz, 1999).

Consumption of proteins has a large metabolic effect because protein consumption increases the thermic effect of food, which increases calorie expenditure postprandially (Weigle et al., 2005; Baba et al., 1999). Thermic effect of food refers to the energy required by the digestion, absorption, metabolism, and storage of food (Nelms and Sucher, 2015). Thermic effect of food is one of three components of energy expenditure, accounting for the least amount of total energy expenditure; it is influenced by both the macronutrient (protein, carbohydrate, or fat) makeup of foods and the amount eaten, and its effects can last up to four hours postprandial (Nelms and Sucher, 2015). The macronutrient protein increases thermic effect of food through requiring more energy to facilitate digestion than fats or carbohydrates (Weigle et al., 2005). The other two forms of energy expenditure that significantly contribute to a person's daily total energy expenditure are the resting metabolic rate, also referred to as resting energy expenditure, and the thermic effect of activity. Resting energy expenditure is the energy necessitated by a body at rest in order for body systems to function (Nelms and Sucher, 2015). Resting energy expenditure makes up the majority of the total energy expenditure, while thermic effect of activity is the most variable contributor to total expenditure—it is the energy expended with any physical work or heat generation that requires muscular initiation (Nelms and Sucher, 2015).

Protein quality describes a food protein's content of essential amino acids as well as its digestibility, or its ability to be absorbed (Millward et al., 2008). Higher quality proteins may affect satiety to a greater degree than lower quality proteins based upon their content of essential amino acids, those involved in the regulation of protein synthesis, protein degradation, insulin secretion/synthesis, and hormone signaling, among other processes (Veldhorst et al., 2009). The amino acid content of various proteins may contribute to food intake through neurochemical signaling (Anderson and Moore, 2004), but amino acid profile may also affect the thermic effect of food through the differences in the ways that the amino acids are oxidized (Veldhorst et al., 2008).

Another factor that coincides with amino acid content and can influence metabolic responses is the digestive actions of proteins (Millward et al., 2008; He and Giuseppin, 2014; Anderson and Moore, 2004). The processes that take place in the gastrointestinal tract involving proteins may affect food intake independently of their amino acid composition (Anderson and Moore, 2004; Hall et al., 2003). Protein type may influence the rate of each protein to be digested and absorbed (Lang et al., 1998), which influences the rate at which amino acids are present in circulation (He and Giuseppin, 2014), which in turn may influence feelings of satiety (Hall et al., 2003). Because of the complex multi-system interactions that regulate appetite, it is more difficult to determine how unique protein types influence satiety than to discover that correlative differences exist among protein sources and satiety, metabolic rate, and postprandial food intake (Millward et al., 2008).

The need for more research on the implications of protein sources on food intake, metabolism, and health is apparent due to the limited or conflicting current knowledge of the effects of various protein sources, as well as the mechanisms by which various protein sources act on metabolism (Anderson and Moore, 2004; Veldhorst et al., 2008; Veldhorst et al., 2009; Lang et al., 1998; Douglas et al., 2015). Therefore, the objective of this study was to further contribute to the research pool through examining the impacts of different protein sources on postprandial metabolism, satiety, and food intake. We hypothesize that higher-quality complete protein isolates (e.g. animal sources of protein) would be more satiating and have a higher thermic effect of food than the incomplete protein isolates (e.g., plant sources of protein).

Materials and Methods

Subject Recruitment and Participation

Subjects were recruited on a voluntary basis in fall 2015 by advertisement in University of Arkansas Newswire (an e-news source for the University), on flyers in University buildings, through social media (e.g. Facebook, twitter), and by word of mouth. All interested potential subjects corresponded via email and were screened by phone. The participants had no health conditions, food allergies/intolerances, and were not prescribed any medications. All participants were non-smokers, were not currently dieting, and were not participating in more than 4 hours of strenuous physical activity per week. Eight adult males (n = 8) ages 18 to 36 were recruited, however, only 4 people were able to participate for the duration of the study as 4 subjects dropped out due to either scheduling issues or difficulties complying with the study protocol. All participants signed and submitted a participant consent form before taking part in the study. Participants were randomly assigned to treatment groups and given coded subject labels to protect participant privacy. Upon completion of the study, subjects received a gift card and a free body composition scan (DXA) as compensation for their participation. The study design was approved by the University of Arkansas' Institutional Review Board (IRB) (protocol #15-07-005).

Study Design

The study was a randomized, crossover design. Participants received each dietary treatment with a one-week washout period (time between the administration of each treatment to control for potential interactions) between treatments. The three treatments included: a beef-sourced protein drink, a pea-sourced protein drink, and a whey-sourced protein drink (refer to Table 1 for compositions

Table 1. Nutrient compositions of protein drink treatments.

	Drink Type		
Nutrient	**Beef**	**Pea**	**Whey**
Kcal Content	275 kcal	275 kcal	275 kcal
Carbohydrate Content	15.4 g	15.5 g	14.9 g
Protein Content	42.3 g	41.2 g	43.2 g
Fat Content	04.5 g	04.3 g	04.6 g
Fiber Content	<1.0 g	03.4 g	01.7 g

and Table 2 for recipes of test drinks). Participants were asked to consume one treatment on each consecutive testing day spaced one week apart.

Participants were asked to refrain from eating at least 8 hours overnight prior to each test day—initial measurements were collected while participants were in a fasted state. Participants arrived at the Food Science Building at the University of Arkansas, Fayetteville, Arkansas between 7:00 AM and 7:30 AM. Upon arrival, standing height and weight were measured; baseline satiety values were recorded using visual analog scales (VAS). Resting energy expenditure was measured using a metabolic cart. Following baseline measurements, participants were provided with the test breakfast beverage. Participants were given 8 minutes to consume the entire beverage. After consumption, participants were asked to refrain from eating for 4 hours. Small amounts of water were permitted according to subjects' thirst. During the 4-hour period, participants' appetites were assessed periodically using VAS scale surveys: at 0, 15, 30, 60, 120, 180, and 240 minutes postprandial. Data using a metabolic cart were also collected at six time points throughout the four hours: at 0, 30, 60, 120, 180, and 240 minutes postprandial. In addition, participants were also asked to record food intake for the following 24 hours beginning at the end of the test day using a provided food diary form, for a total of 3 food records per participant.

Measurements and Data Analysis

Height, Body Weight, and Body Mass Index (BMI). The height of each participant was measured to the nearest 0.1 cm using a stadiometer while barefoot, in a freestanding position. Body weight was measured at each visit for each subject (without shoes) to the nearest 0.05 kg using calibrated balance scales. Body mass index was calculated as weight (kg) divided by height (m) squared.

Appetite Assessment. Participants were asked to rate their perceived hunger, fullness, strength of desire to eat, desire for a snack, amount of prospective food desired, cravings for salty foods, and cravings for sweet foods using VAS spanning 100 mm with opposing anchors (e.g. "extremely hungry" to "not hungry at all"). Appetite was measured periodically at 0, 15, 30, 60, 120, 180, and 240 minutes postprandial.

Resting Metabolic Rate and Thermic Effect of Feeding. Resting metabolic rate was measured with a TrueMax® 2400 metabolic cart (Parvo Medics, Sandy, Utah) and used to find the thermic effect of food, the rate of carbohydrate oxidation (KCHO), and the rate of fat oxidation (KFAT). Indirect calorimetry, using the ventilation hood technique, was measured in 15-second increments after rest periods while in the supine, reclined position. A canopy hood was placed over each participant and breath-by-breath analysis was conducted for 30 minutes (at time point 0) or for 20 minutes (at each of the following time points across 240 minutes). Thermic effect of food was determined by assessing the difference in resting metabolic rate immediately before and 30, 60, 120, 180, and 240 minutes after the consumption of the test protein drinks.

Dietary Assessment. The energy and macronutrient composition of test drinks and 24-hour dietary records were analyzed for each participant using Genesis R&D nutrient analysis software (ESHA Research, Salem, Ore.) and information was organized by test drink.

Statistical Analysis. Repeated measures analysis of variance (ANOVA), two-way ANOVA and *t*-tests were used to compare the differences among the three protein treatments' effects on metabolism, hunger, satiation, and cravings. In order to analyze the effects of the protein drinks across the 4-hour test period, net incremental area under the curve (niAUC) was calculated using the trapezoidal rule; niAUC was then analyzed using one-way ANOVA. GraphPad Prism Software v. 6.0 (La Jolla, Calif.) was used for all data analysis and figure production.

Results and Discussion

Participant Characteristics

A total of four participants completed the study in its entirety. Table 3 shows the baseline anthropometric measurements and other specific characteristics of participants.

Table 2. Recipe for protein drink treatments.

	Drink Type		
Ingredient	Beef	Pea	Whey
Water added	385.0 mL	385.0 mL	385.0 mL
Powder mix added	47.6 g	75.6 g	58.8 g
Canola oil added	4.5 g	-	2.0 g
Cane sugar added	12.0 g	-	8.0 g

Metabolic Measurements

Resting Energy Expenditure and Thermic Effect of Food. The pea treatment had a significantly higher resting energy expenditure than the beef protein treatment (P = 0.02, Fig. 1). The resting energy expenditure niAUC for beef was 8% lower than the niAUC for pea and 5% lower than the niAUC for whey. There were significant differences in thermic effect of food between pea and whey and between beef and whey ($P < 0.05$, Fig. 2). The niAUC for thermic effect of food found no differences among treatments, though the niAUC for whey was 77% higher than the niAUC for beef and 43% higher than pea.

Carbohydrate Oxidation and Fat Oxidation. There was no significant difference between treatments for KCHO (Fig. 3). There was a significant difference in KFAT between the rate of whey over the rate of pea ($P < 0.05$, Fig. 4).

Appetite Assessments

Perceived Hunger and Fullness. Perceived hunger increased and fullness of the participants measured by VAS scale decreased over time (Fig. 5). However, there was no difference in hunger between protein treatments. There was a significant difference in perceived fullness following the beef treatment compared to the pea and whey treatments ($P < 0.05$, Fig. 5).

Strength of Desire to Eat and Prospective Food Consumption. There was no difference in desire to eat between the three treatments. However, perceived desire for a snack was higher with beef protein compared to whey protein ($P < 0.05$, Fig. 6). For prospective amount of food desired, there was a significantly greater desire ($P < 0.05$) to eat more food following the beef protein than there were following the pea or whey protein (Fig. 7).

Table 3. Participant characteristics.

Characteristic	Value
Age, years[a]	21.75 ± 2.63
Height, cm	179.07 ± 7.90
Weight, kg	100.58 ± 24.67
BMI	31.57 ± 8.75
Fat Mass, kg	26.87 ± 18.87
Fat Free Mass, kg	70.46 ± 10.60
Ethnicity	
Black	2
White	1
Other	1
Total	4

[a] Age, height, weight, body mass index (BMI), Fat Mass, and Fat Free Mass are expressed as mean ±SEM.

Table 4. Dietary intake following treatments.

Nutrient Data	Beef	Pea	Whey
Energy, kcal[a]	3557.20 ± 1745.70	2736.80 ± 0451.80	3071.60 ± 1740.50
CHO, g	0441.46 ± 0253.06	0325.96 ± 0078.96	0340.14 ± 0174.10
% Kcal from CHO	50%	48%	45%
PRO, g	0127.47 ± 0049.69	0126.25 ± 0035.92	0114.01 ± 0068.41
% Kcal from PRO	14%	18%	15%
FAT, g	0139.30 ± 0074.31	0102.58 ± 0027.64	0133.04 ± 0111.53
% Kcal from FAT	36%	34%	40%

[a] Energy, carbohydrate (CHO), protein (PRO), and FAT are expressed as mean ± standard error of mean.

Perceived Salty/Sweet Cravings. There was no difference in cravings for salty and sweet foods between protein treatments.

Recorded Dietary Intakes

The beef protein treatment relates on average with the highest postprandial intake of calories and grams of each

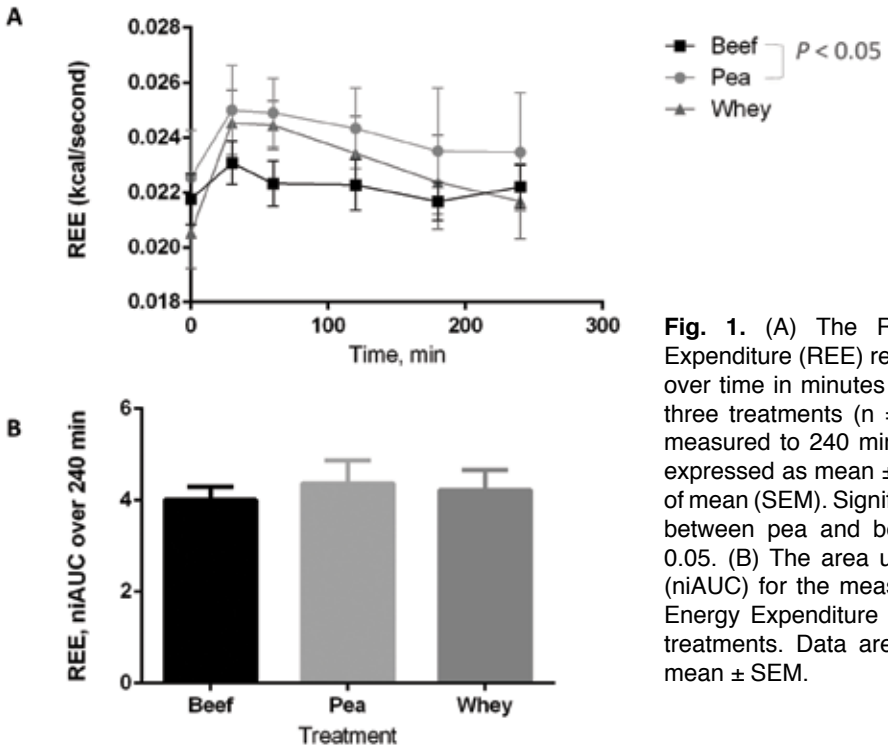

Fig. 1. (A) The Resting Energy Expenditure (REE) results averaged over time in minutes for each of the three treatments (n = 4). Time was measured to 240 minutes. Data are expressed as mean ± standard error of mean (SEM). Significant difference between pea and beef where $P < 0.05$. (B) The area under the curve (niAUC) for the measure of Resting Energy Expenditure for each of the treatments. Data are expressed as mean ± SEM.

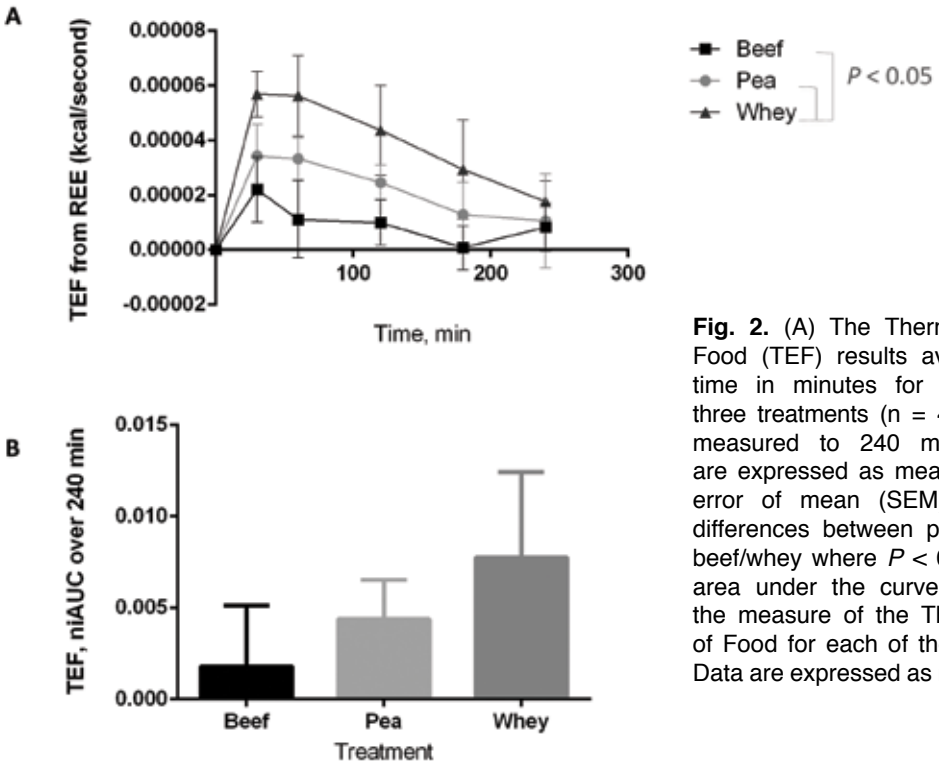

Fig. 2. (A) The Thermal Effect of Food (TEF) results averaged over time in minutes for each of the three treatments (n = 4). Time was measured to 240 minutes. Data are expressed as mean ± standard error of mean (SEM). Significant differences between pea/whey and beef/whey where $P < 0.05$. (B) The area under the curve (niAUC) for the measure of the Thermal Effect of Food for each of the treatments. Data are expressed as mean ± SEM.

macronutrient (Table 4 shows the average consumption of kcal, carbohydrate, protein, and fat in the 24-hour period following each protein treatment and the percentage of kcal from each macronutrient within each treatment category). The beef protein treatment was followed, on average, by an intake of 485 more calories than the whey treatment and 820 more calories than the pea treatment, though the standard deviations from the means were

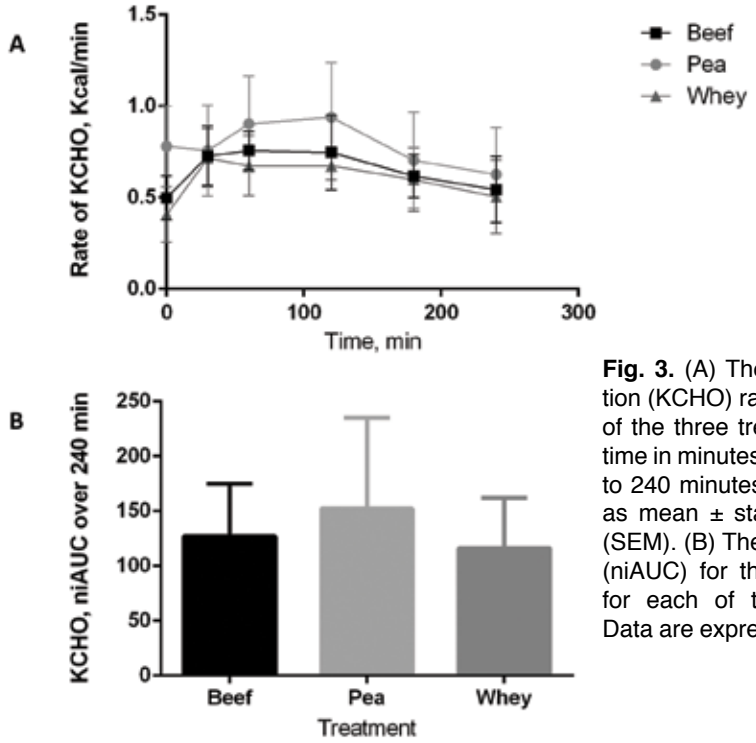

Fig. 3. (A) The carbohydrate oxidation (KCHO) rates averaged for each of the three treatments (n = 4) over time in minutes. Time was measured to 240 minutes. Data are expressed as mean ± standard error of mean (SEM). (B) The area under the curve (niAUC) for the measure of KCHO for each of the three treatments. Data are expressed as mean ± SEM.

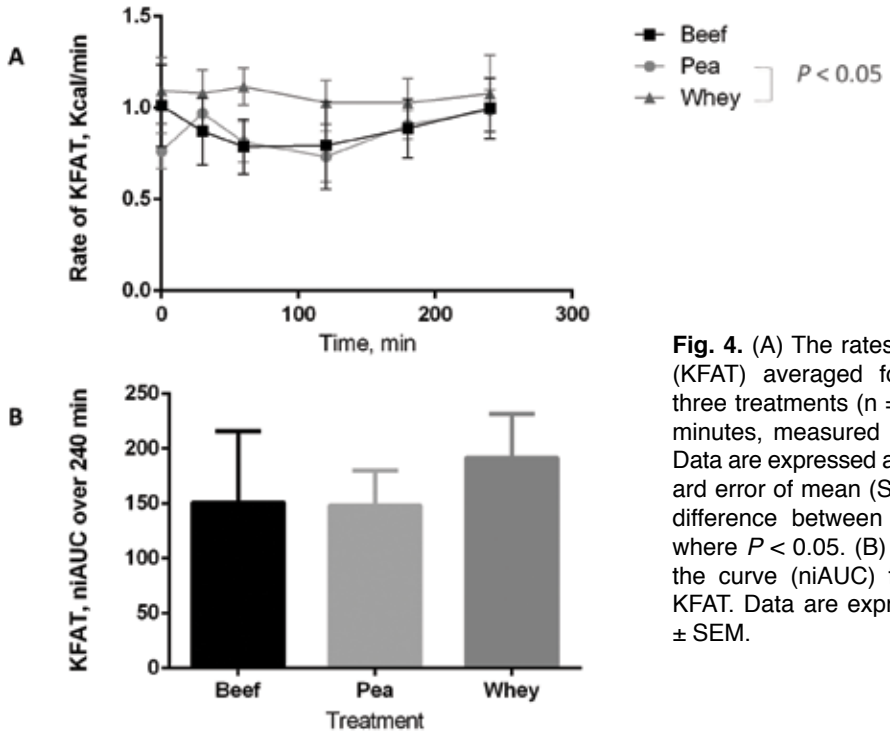

Fig. 4. (A) The rates of fat oxidation (KFAT) averaged for each of the three treatments (n = 4) over time in minutes, measured to 240 minutes. Data are expressed as mean ± standard error of mean (SEM). Significant difference between whey and pea where $P < 0.05$. (B) The area under the curve (niAUC) for the rates of KFAT. Data are expressed as mean ± SEM.

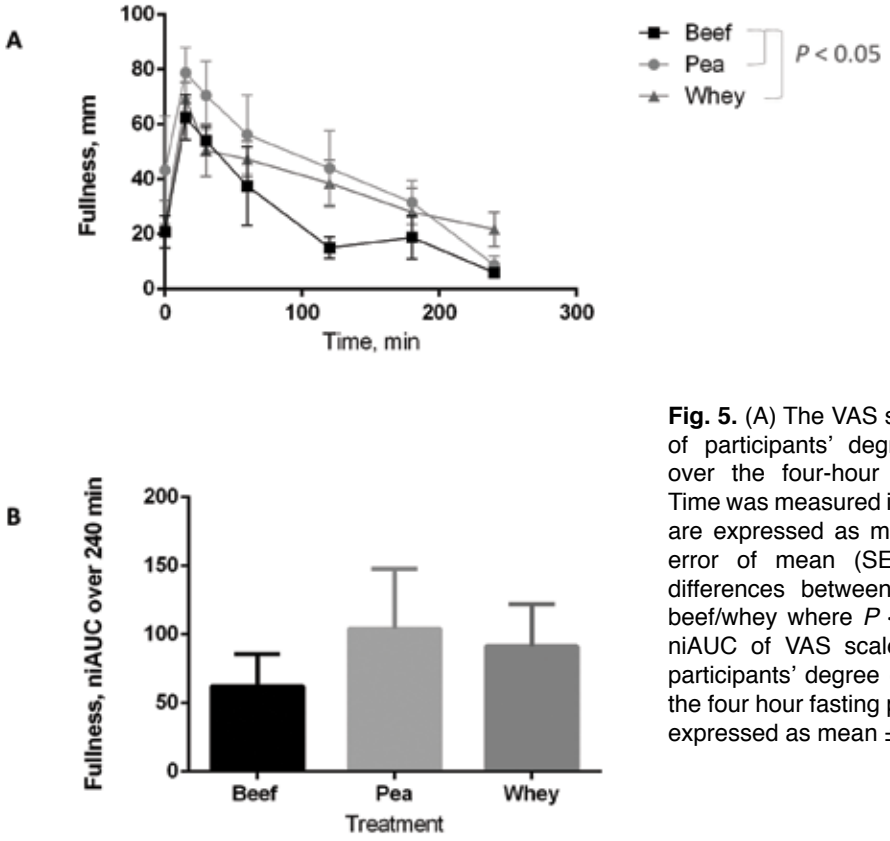

Fig. 5. (A) The VAS scales' measure of participants' degree of fullness over the four-hour fasting period. Time was measured in minutes. Data are expressed as mean ± standard error of mean (SEM). Significant differences between beef/pea and beef/whey where $P < 0.05$. (B) The niAUC of VAS scales' measure of participants' degree of fullness over the four hour fasting period. Data are expressed as mean ± SEM.

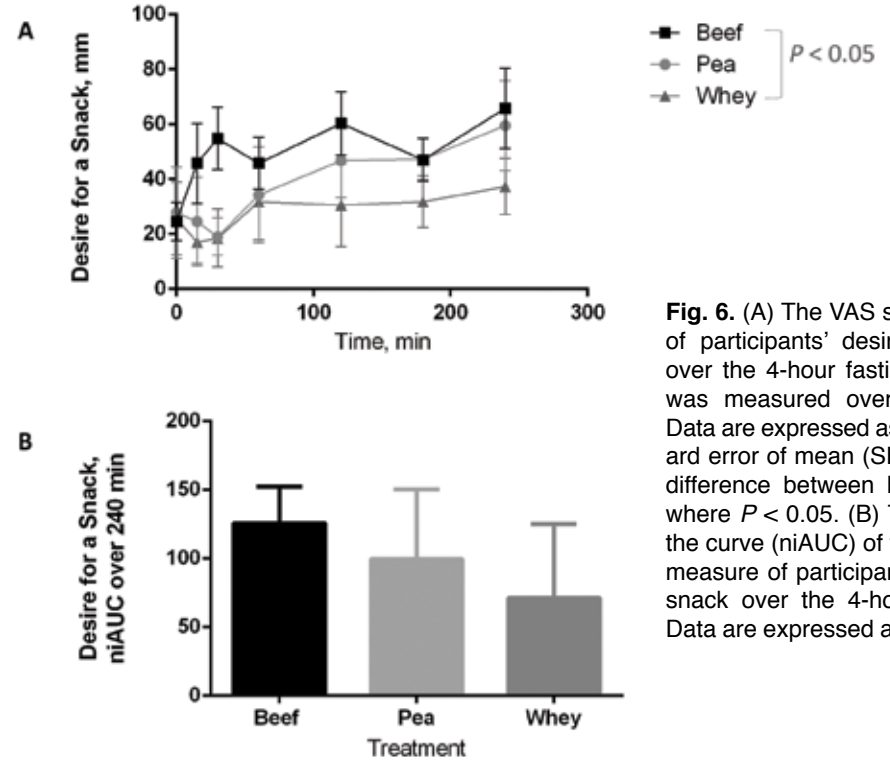

Fig. 6. (A) The VAS scales' measure of participants' desire for a snack over the 4-hour fasting period; time was measured over 240 minutes. Data are expressed as mean ± standard error of mean (SEM). Significant difference between beef and whey where $P < 0.05$. (B) The area under the curve (niAUC) of the VAS scales' measure of participants' desire for a snack over the 4-hour test period. Data are expressed as mean ± SEM.

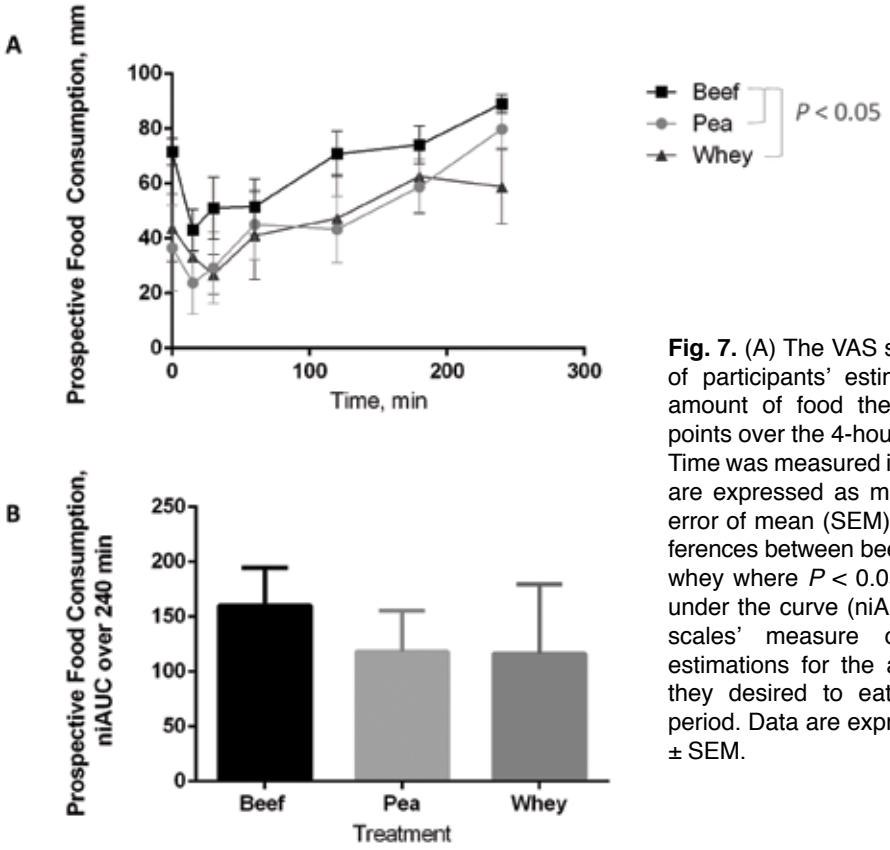

Fig. 7. (A) The VAS scales' measure of participants' estimations for the amount of food they could eat at points over the 4-hour fasting period. Time was measured in minutes. Data are expressed as mean ± standard error of mean (SEM). Significant differences between beef/pea and beef/whey where $P < 0.05$. (B) The area under the curve (niAUC) of the VAS scales' measure of participants' estimations for the amount of food they desired to eat over the test period. Data are expressed as mean ± SEM.

large. Fat intake following the beef protein contributed an average of nearly 36% of calories from fat while the intake of calories from fat after ingestion of the pea and whey proteins were similarly 34% and 40%, respectively. The postprandial intake of participants following each of the three protein treatments was statistically similar.

Discussion

The large range of protein choices commercially available and the great variation in food selection, dietary supplementation, and overall protein intake among modern consumers, normal weight or otherwise, support our research interest in determining the metabolic effects of different protein sources (Hall et al., 2003).

This study explored the potential for several varying effects among individual protein sources consumed as isocaloric test drinks (comprised of near identical macronutrients), on the metabolisms of healthy young adult males. It was our hypothesis that "complete" protein would have the greatest metabolic effect regarding resting energy expenditure and thermic effect of food based upon current research (Millward et al., 2008), and "incomplete" protein would be less satiating than "complete" protein (Millward et al., 2008). Results from this study revealed that beef protein overall was less satiating and increased metabolic rate to a lesser degree than whey or pea proteins. However, minimal significant differences among beef, pea, and whey isolate proteins were found, though relationships were detected that could have larger implications in a more expansive study.

The measures of resting energy expenditure and thermic effect of food were affected by protein source, though the treatments would need a repeat testing to look for greater significance as there were discrepancies present. Thermic effect of food seemed to be significantly affected by whey over pea and beef in some tests, and resting energy expenditure was significantly raised with pea consumption above the consumption of beef protein in few but not all tests as well. In a recent study, whey was the leading protein found to increase energy expenditure through resting energy expenditure and thermic effect of food to a greater degree than casein or soy (Acheson et al., 2011). The perception of fullness was significantly affected by protein source in our study, with beef being significantly less satiating than pea or whey. The reciprocal measure of perceived hunger found no significant differences, though overall beef correlated with greater feelings of hunger and lesser feelings of fullness. In similar satiety

studies comparing milk/soy proteins and amount of protein, a whey treatment was found to correlate with the greatest feelings of hunger and least feelings of fullness (Acheson et al., 2011), while a higher amount of protein led to the greatest feelings of fullness (Leidy et al., 2013).

Protein source could also be an important factor when considering connections between physiological/neural responses post-ingestion. The differences in perceived strength of desire for food showed no statistical significance, but the perceived desire for a snack and the amount of prospective food consumption in our study were significantly greater following the beef treatment than following the whey treatment (or the pea treatment for the amount of prospective food consumption). Similar protein studies have found prospective food consumption to be greatest following ingestion of whey protein compared to casein and soy proteins (Acheson et al., 2011).

With regard to the dietary intake of study participants following each study day, participants on average consumed a similar amount of calories, carbohydrates, protein, and fat in the 24 hours following the treatment of beef protein as the treatments of pea and whey proteins. Current research has also found protein breakfasts of varying protein amounts and sources to have similar daily intakes, though high fat snacks were more limited when test breakfasts were higher in protein (Leidy et al, 2013), reinforcing the idea that the presence of protein at breakfast may be more influential than the amount or type of protein.

The KCHO and KFAT rates among the treatments were not of statistical significance. However, the rate of KCHO following the pea test drink was consistently higher than the rates of KCHO after consumption of beef protein or whey protein. Though the test drinks were nearly identical in all macronutrient content, carbohydrate metabolism was elevated in this study following pea protein ingestion. This finding (among others) may be attributed to the unequal distribution of the fiber content of the test drinks, a value greatest in the pea treatment (Douglas et al., 2015; Lang et al., 1998; Latner and Schwartz, 1999). If fiber content is correlated to the elevated rate of KCHO, it is interesting to note how such small differences in fiber may have manipulated the observed rates. For KFAT rates, fat metabolism was consistently highest after the whey treatment with only a slight difference between the rates of pea and beef seen graphically (Fig. 4a,b). The elevated rate of KFAT following the whey treatment is consistent with recent research that found the rate of KFAT to be significantly higher following a whey treatment than after treatments of casein and soy proteins (Acheson et al., 2011).

Cravings for salty versus sweet foods throughout the fasting period showed no statistical significance among the different proteins, suggesting that the taste of food desired following protein ingestion may not be as affected as the type of macronutrient desired. However, it was interesting to note that the recorded cravings for salty foods were higher in general than the recorded cravings for sweet foods. Sweet tasting foods frequently contain significant amounts of fat as well as refined sugars. Further testing of cravings may support the current evidence that consuming high amounts of protein reduces cravings for fatty foods and cravings for food in general (Latner and Schwartz, 1999).

Limitations of the study include the small sample population (n = 4). Had more young adult males been able to participate within the window of the study, the correlations that polarized the beef, pea, and whey protein treatments might have been more statistically significant. Also, food records as a quantitative way of assessing postprandial caloric and macronutrient intake are often found to be inaccurate due to their self-assessing nature. In addition, this study focused on testing proteins that were in isolate powdered form and ingested as a drink. Studies testing non-isolate proteins, solid foods, individual amino acids, or mixed meals may have varying metabolic results (Douglas et al., 2015). The amino acid profiles of the tested proteins (beef, pea, whey) may have greatly attributed to our results, as well as the amount of protein tested (Douglas et al., 2015). Lastly, generalizations across genders, ages, and BMI categories for our observations cannot be made since the population examined was limited to young adult males (He and Giuseppin, 2014).

Across all measurements of the study, the observation of beef protein to be less satiating and to have a lesser effect on raising metabolism, as well as the observation for whey protein to be more satiating, is prevalent, but not significant. These data suggest that protein source (animal versus plant) is not a predictor of postprandial EE and appetite response. As statistically significant differences were not common despite clearly observed graphical differences within our small, tested sample, it is recommended that protein sources related to degrees of satiation and rates of energy expenditure should be more extensively studied, with particular attention to beef/whey proteins and fiber content. Other unstudied isolate proteins at different protein loads are in need of testing, as well as individually ingested amino acids. Further research of potential correlations among specific proteins and their subsequent effects on energy metabolism, satiety, and postprandial food intake is essential to understanding the unique metabolic properties of particular protein sources and their role in promoting healthy appetites and active metabolisms.

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Using precision agriculture field data to evaluate combine harvesting efficiency

Justin H. Carroll[*], *Don Johnson*[†], *Jeff Miller*[§], *and Kristofor Brye*[‡]

Abstract

Soybeans must be harvested during a limited time period using expensive combines and associated equipment. Maximizing combine field efficiency, the ratio of the actual harvesting capacity to theoretical harvesting capacity, is an important objective of machinery managers. Spatial and temporal yield data from a 2012 CaseIH 8120 Axial-Flow combine equipped with a 9 meter MacDon D-65 Draper header and the Case-IH Advanced Farming System (AFS) yield monitoring system were used to examine field efficiency when harvesting soybean in three Arkansas Delta irrigated soybean fields during the 2015 season. Time efficiencies (TE) in the three fields ranged from 72.9% to 85.8% (mean = 80.9%, standard deviation (SD) = 9.6%); width efficiencies (WE) ranged from 96.7% to 98.8% (mean = 97.6%, SD = 1.6%); and overall field efficiencies (FE) ranged from 70.4% to 84.8% (mean = 79.0%, SD = 9.7%). Contrary to expectations, neither row length nor unadjusted yield was significantly correlated ($P < 0.05$) with time efficiency, width efficiency, or field efficiency. Time efficiency explained 90.5% ($sr^2 = 0.905$) of the unique variance in field efficiency, while WE explained only 1.6% ($sr^2 = 0.016$) of the variance in FE when controlling for the effects of TE. Results indicated that the use of geo-referenced field and performance data can be helpful in evaluating combine performance and efficiency.

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Meet the Student-Author

Justin Carroll

I am from Brinkley, Arkansas and graduated from Brinkley High School in 2012. In 2015, I graduated from the University of Arkansas with a Bachelor of Science degree in Agricultural Education, Communication, and Technology. This spring, I began pursuing a Masters Degree in Agricultural and Extension Education.

During my undergraduate career, I was a member of the Razorback chapter of Collegiate Farm Bureau, the Xi chapter of Kappa Sigma, and Ducks Unlimited. Through my honors thesis research, I gained valuable research experience and learned the importance of student-mentor communication in accomplishing large tasks.

I would like to thank Don Johnson for supporting me and providing me with his guidance while I completed my honors research. I would also like to thank Jeff Miller and Kristofor Brye for providing valuable input, advice, and serving on my committee. I am also honored to have received funding for this research from the Dale Bumpers College of Agricultural, Food, and Life Sciences.

Introduction

In the next 50 years farmers around the world will have to feed more people than they have in the previous 100 years (Arkansas Farm Bureau, 2014). To help accomplish this task, farmers will have to reduce costs, while increasing the field efficiencies of their machinery by making smarter machinery management decisions through the use of precision agriculture practices.

Machinery costs account for 35-50% of total fixed costs, so using machinery more efficiently can provide for significant savings for the farmer (Yule et al., 1999). Knowing field efficiency (FE) is crucial in maximizing profit in association with how efficiently fuel is being used, number of working days during harvest, and ultimate timeliness in the field. In the case of time costs, farmers have a time window during certain dates of the year in which to harvest their crop optimally, this is referred to as the base harvest period. After that optimal time, there is a yield loss each week thereafter. For soybeans the "excess harvest loss expected" is one bushel for an acre harvested in the first week after the base harvest period, two bushels in the second week and so on (Short and Gitu, 1991). Determining the FE of the combine is imperative in order to know how many hours of work it will take to make sure the crop is harvested during the optimal time and yield loss is minimized or non-existent in order to increase profits.

Agricultural machines' FEs have a significant effect on the effective field capacities of machinery, which in turn impact the overall cost of production (Pitla et al., 2015). Effective field capacity is defined as the actual rate of crop processed in a given time (ASAE, 2005). Field efficiency is defined as the ratio of effective field capacity to theoretical field capacity expressed as a percentage, with effective field capacity being the actual rate of land or crop processed in a given time and theoretical field capacity referring to the rate of performance of a machine functioning 100% of the time at a given speed using 100% of its theoretical width (ASAE, 2005).

Computationally, FE is the product of time efficiency (TE) and width efficiency (WE) (Field and Sollie, 2007). Time efficiency is the ratio of productive field time to total field time (i.e., the ratio of actual harvesting time to total operating time). Width efficiency is the ratio of the actual machine width used to the functional operating width of the machine (Hunt, 2001).

Field efficiencies for a self-propelled combine range from 65-80%, with typical combines achieving 70% (ASAE, 2011). Efficiency varies due to a variety of factors including turning time, speed, machine width, row length, and crop yield (Hunt, 2001). Crop yield affects the field efficiency of a combine when standard or typical field speeds are used to calculate theoretical field capacities, with greater yields usually resulting in reduced travel speed (Grisso et al., 2002).

Row length may also affect FE for operations, such as combine harvesting, where the machine cannot perform its intended function while turning at row ends; FE would be expected to increase with increased row length. According to Grisso et al. (2002), if implement width stays the same and row lengths double, field efficiency improves because the proportion of implement operating time increases with respect to its turning time.

Harrigan (2003) conducted time-motion studies of corn silage harvesting operations on seven Michigan dairy farms and reported a mean TE of 85% when truck- or tractor-drawn transport vehicles were driven alongside the harvester. Unproductive time consisted of time spent in turning the harvester in the headlands and switching transport vehicles. Niehaus (2014) used spatial data to evaluate the corn harvesting operation on an Iowa grain farm and reported an overall TE of 62.4%; with 16.1% of total time spent in machine idling, 9.1% in in-field or road travel, 9.3% in turning within field headlands, and 2.9% unloading grain while not harvesting.

The objectives of this study were to determine (a) the width efficiency, time efficiency, and overall field efficiency of a combine harvesting soybeans on a typical Arkansas Delta farm, and (b) the relationship between row length, yield, WE, TE and FE.

Key Terms

- Advanced Farming Systems (AFS) are factory installed machine technology capable of recording yield and spatial data and monitoring machine conditions.
- Field efficiency is the ratio of effective field capacity to theoretical field capacity expressed as a percentage, with effective field capacity being the actual rate of land or crop processed in a given time and theoretical field capacity referring to the rate of performance of a machine functioning 100% of the time at a given speed using 100% of its theoretical width (ASAE, 2005).
- Row length is the effective length, in meters, that the combine traveled in one pass through the field.
- Crop yield is the amount of crop harvested over a given area. Kilograms per hectare is the unit of measurement used.
- FarmLogic is farm record keeping software.

Materials and Methods

The field efficiency of a 2012 CaseIH 8120 Axial-Flow combine (Fig. 1) harvesting with a 9-meter MacDon D-65 Draper header was tested. Since one of the independent variables was crop yield, the onboard AFS was used, equipped with an AFS Pro 600 Model display and an AFS 262 GPS receiver (Fig. 2), to record the unadjusted (wet basis) yield. The AFS 262 GPS receiver used Wide Area Augmentation System (WAAS) frequency corrected from a reference station in Memphis, Tennes-

Fig. 1. 2012 Case-IH 8120 Axial-Flow combine used in harvesting soybean.

Fig. 2. AFS Pro 600 Display (left) and AFS 262 Receiver (right).

see with 15-30 cm accuracy. To achieve accuracy in yield readings, a field technology consultant for Eldridge Supply in Brinkley, Arkansas, calibrated the moisture sensor using fields harvested prior to the study. The moisture sensor compartment was hand cleaned and checked before harvest began each day by cutting a sample in the field perimeter. The accuracy of the AFS was checked by comparing AFS readings to moisture of the previously cut samples and checking that sample for the accuracy to affirm the AFS readings were correct. Accuracy was checked against a desktop moisture machine at local grain bins by inserting the previously cut sample into the machine and noting the readout, which matched the AFS readout.

To achieve operator uniformity, the same operator, with more than 30 years of harvesting experience, harvested each field. The operator was informed that the travel pattern should be consistent across all three fields and that edges should be cut first. The combine was lubricated at the beginning of each day, and hydraulic and engine oil levels were checked to ensure proper machine function. Prior to harvest each day, the on-board AFS records were reviewed for correct farm and field name to ensure data was being stored under the correct name for the current field.

The AFS hardware and software collected and stored georeferenced harvest data including spatial position, field travel speed, mass grain flow, grain moisture, pass-to-pass machine width, total operating time, and productive operating time data were logged automatically at a rate of 1-Hz.

The three fields (Fig. 3) selected for data collection were located southeast of Brinkley, Arkansas and northwest of Moro, Arkansas. The fields were owned and farmed by Jimel Farms Inc. All three fields were farmed in a conventionally tilled corn-soybean rotation for four years prior to the study. Fields varied in size from approximately 49 ha (hectares) to approximately 91 ha and were relatively rectangular in shape. Each field was divided into four approximately sized replicates post-harvest using ArcGIS software.

Fields of different lengths, ranging from approximately 280 m to 420 m, were selected so the effect of row length on FE could be evaluated; the exact field length of each replicate was measured using the measurement tool in FarmLogic. The soils in each field were similar, with each having a significant amount of Foley-Calhoun-Bonn complex, silt loam, and Grenada silt loam. Fields one and three were leveled throughout, while field two had a small ridge running through the middle and sloping off to either side. The three fields were planted with conventional soybeans in the 4.6 maturity group. Soybean was planted on 60-inch beds with 15-inch spacing between each row of soybean and three rows per bed.

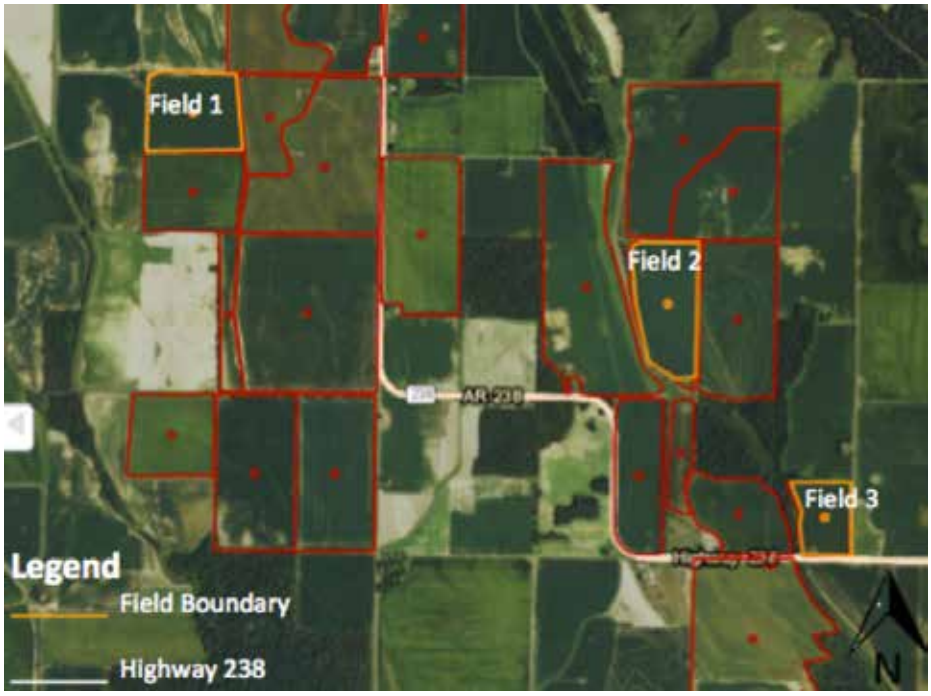

Fig. 3. Aerial map showing fields used in combine harvesting study.

The headlands in each field were harvested prior to initiation of this study. In addition, a grain cart was driven in the field alongside the combine and the combine was unloaded on the go as is customary on this farm.

Several assumptions were made during the study in order to adhere to reasonable harvest dates. The AFS technology was calibrated prior to data collection, so it was assumed that the AFS technology on the combine was accurate in order to collect useable data. Calibration involved harvesting samples of grain and weighing them with a scale-equipped wagon in order to input actual weights into the combine so that the AFS could average those weights with those it recorded during harvesting. The moisture measurements reported from the desktop moisture machine were assumed to be accurate so that the on-board moisture sensor readings were confirmed. Since the same operator was involved in all data collection it was assumed that all patterns involving driving technique were consistent. Also, even though the fields were not all planted on exactly the same date, it was assumed that all three fields had optimal periods for the crop to grow.

Once the data were collected, a FieldPro for Greenway Equipment in Brinkley, Arkansas, used AgStudios by Mapshots to convert the data into a viewable format as point data and shape files. The data set was imported into ArcGIS and separated into four polygons per field for replication purposes. The data within each point in each polygon were imported into Microsoft Excel and TE (productive time/total time) and WE (pass-to-pass machine width/total machine width) were calculated. Finally, the means for all study variables were calculated for each replication by field. These mean values were then imported into SAS® 9.3 for statistical analysis using descriptive and correlational statistics such as Pearson correlation and squared semipartial correlation. Computationally, because FE is the product of WE and TE, a linear combination of these two variables would be expected to explain 100% of the variance in FE. However, the relative importance of WE and TE in explaining the variance in FE was not known; therefore squared semipartial correlations (sr^2) were calculated to determine the unique variance in FE accounted for by WE and TE when statistically controlling for the effects of the other variable (O'Rourke et al., 2005).

Results and Discussion

Descriptive statistics for plot size, row length, grain moisture, unadjusted and adjusted yields are presented, by field, in Table 1. Mean row lengths for the three fields ranged from 277 m to 423 m and mean unadjusted yields ranged from 3416.2 kg/ha to 4281.8 kg/ha. Adjusted to standard 13% moisture content, mean yields ranged from 3648.3 kg/ha to 4371.9 kg/ha.

Table 2 provides summary statistics for various combine performance measures by field. Mean field speeds ranged from 4.0 to 6.0 km/h with an overall mean field speed of 4.8 km/h. The combine was operated at nearly its full working width in each field, with mean WEs of between 97.4% and 98.8% and an overall mean WE of 98%. Mean TEs ranged from 73% to 85.8% for an overall mean TE of 80.9%. The resulting mean FEs ranged from 70.4% to 84.8% (Field 1) for an overall FE of 79%.

There were no statistically significant bivariate correlations between either row length or yield and any measure of combine efficiency (Table 3). There was a significant positive correlation (r = 0.99) between TE and FE; however the correlation between WE and FE (r = 0.31, P = 0.33) was not statistically significant. There was a significant positive correlation (r = 0.97) between row length and unadjusted yield. However, this relationship was judged to be spurious and was disregarded, as there was no empirical or theoretical rationale for an association between the length of a field and yield. There was a significant positive correlation (r = 0.63) between grain moisture and field speed. This relationship was thought to be due to the fact that less grain shattering in higher moisture fields allowed for faster field speed despite higher yields. There was a significant positive correlation (r = 0.96) between grain moisture and unadjusted yield. This correlation was not considered important because higher moisture means higher weight of crop and the combine reads yield by weight of crop.

The results indicated TE was the most important predictor, explaining 90.5% (sr^2 = 0.9046) of the unique variance in FE; WE explained only 1.6% (sr^2 = 0.0163) of the variance in FE when controlling for TE. Both coefficients were statistically significant (P < 0.0001). No significant relationship occurred between row length, unadjusted yield, WE, and FE in the study.

Table 1. Means and standard deviations (SD) for field and yield variables by field (n = 4).

	Field					
	No. 1 (14.91 ha)		No. 2 (15.86 ha)		No. 3 (8.15 ha)	
Variable	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Plot size (hectares)	3.72	0.37	3.96	1.16	2.04	1.35
Row length (m.)	423.98	4.50	277.67	96.74	347.47	0.00
Grain moisture (%)	11.41	0.20	8.90	0.07	9.74	0.11
Unadjusted yield (kg/ha)	4281.77	1.39	3416.27	1.30	3784.8	1.03
Adjusted yield (at 13% moisture content)	4371.89	1.42	3648.29	1.36	3942.84	1.07

Table 2. Means and standard deviations (SD) for combine field performance variables by field number (n = 4).

	Field					
	No. 1 (14.91 ha)		No. 2 (15.86 ha)		No. 3 (8.15 ha)	
Variable	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Field speed (km/h)	5.94	0.35	4.80	0.09	3.99	0.33
Working width (m)	9.04	0.23	8.91	0.10	8.84	0.68
Width efficiency (%)	98.85	0.76	97.40	0.00	97.70	0.02
Productive time (min)	35.78	1.24	46.72	4.52	25.52	8.12
Total time (min)	42.03	5.16	55.67	5.45	34.35	6.86
Time efficiency (%)	85.85	7.74	83.95	2.60	72.90	11.90
Field efficiency (%)	84.86	7.51	81.78	2.50	70.40	11.40

Note: Means based on four replications per field.

Table 3. Correlations between row length, unadjusted yield, grain moisture, field speed and combine efficiencies.

Variable	Row length	Unadjusted yield	Grain moisture	Field speed	TE	WE	FE
Row length	1.00	0.97*	0.96*	0.54	0.08	0.37	0.13
Unadjusted yield		1.00	0.96*	0.61*	0.18	0.34	0.21
Grain moisture			1.00	0.63*	0.15	0.48	0.21
Field speed				1.00	0.80*	0.54	0.85*
TE					1.00	0.18	0.99*
WE						1.00	0.31
FE							1.00

*P < 0.05. TE = time efficiency, WE = width efficiency, FE = field efficiency.

The study's results led to several conclusions regarding WE, TE, and FE. Width efficiency was found to be consistent and high (>97.4%) and it was believed to be the result of a function of fit between header width (30 feet) and planting system. Width efficiency would likely be lower for crops using a drill-seeded planting system because there is a certain amount of header overlap practiced in every harvesting pass of drill-seeded crops. Width efficiency caused little variation in FE (r = 0.31) in the planter seeded cropping system used in this study.

Time efficiency was lower than WE and was more variable both within and between fields. The cause of this finding could not be determined from the data collected. Mean FEs range from 70.4% to 84.9%, which is equal to or higher than typical FE, which ranges from 65% to 80% (ASAE, 2011). Time efficiency primarily limited FE because TE was the main factor in calculating FE in the study. Time efficiency alone explained 90.5% of the unique variance in FE, while WE only explained 1.6% of the unique variance in FE. Lack of variance in WE limited its effect on FE. Further research is suggested to identify specific factors affecting TE, as TE plays a major role in achieving typical FE. Shamshiri et al. (2012) calls these factors "non-productive" time and they include turning time at row-ends, driver breaks, equipment adjustment, and machine cleaning. Identifying specific factors affecting TE will allow farm managers to make better decisions in the field so that they can increase overall FE, and in turn increase productivity.

The study's findings related to row length and yield differ from the findings of Grisso et al. (2002). Where Grisso et al. found that higher yield would decrease FE and longer row lengths, when width is held constant, would increase FE, the study found no significant relationship regarding yield, row length, and FE. Difference in methods used may explain the different findings related to yield. In their study, Grisso et al. (2002) used standard field speeds to calculate theoretical field capacity; this study used actual mean field speed in each field to calculate theoretical field capacity.

Extraction and conversion of machine data was one of the difficulties involved in this study, specifically, the compatibility of data and data processing programs. Not all programs can process data from any precision agriculture provider. This study recommends that precision agriculture vendors work to provide more readily available and user-friendly data for farmers, so that they can easily use it to make more informed machinery management decisions.

Based on the high overall high WE in the study, it is recommended that farmers align their header width used in harvesting with their row and bed spacing used while planting. Overall this study concluded that time losses should be limited while harvesting in order to increase TE, which in turn increases overall FE. Therefore, precision agriculture data collected while harvesting can be used to evaluate performance and is a basis for making more informed machinery management decisions.

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Calcium and magnesium absorption and retention by growing goats offered diets with different calcium sources

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Abstract

Calcium addition is necessary in order to balance the high phosphorus concentrations that are characteristic of high-concentrate ruminant diets. However, calcium sources differ in their bioavailability. Our objective was to determine apparent calcium and magnesium absorption and retention in goats offered diets containing different sources of calcium. Spanish-Boer goats (n = 18; 19.6 ± 1.88 kg) were stratified by body weight (BW) and sex and randomized to dietary treatments consisting of Purina Antlermax 16 containing either calcium carbonate (CC), Calmin (CM) or Milk Cal (MC). Goats were adapted to a control, corn-based high-concentrate diet on pasture and then moved to individual 1.0 × 1.5-m pens with plastic coated expanded metal floors, and adjusted to their respective diets along with removal of hay from the diet over a 7-d period. Goats were then offered their respective diets at a total of 2% of BW in equal feedings at 8:30 AM and 5:00 PM for an additional 14-d adaption period to diet and facilities followed by a 7-d collection of total urine and feces. Data were analyzed using PROC MIXED of SAS. Calcium and magnesium intake were not different ($P \geq 0.12$) among diets. Calcium and magnesium apparent absorption and retention (g/d and % of intake) were greatest ($P < 0.05$) in goats offered CC and did not differ ($P \geq 0.20$) between goats offered the CM and MC diets. Therefore, calcium and magnesium were more available for goats from the diet containing calcium carbonate compared with diets containing Calmin and Milk Cal.

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Meet the Student-Author

Jennifer Long

I am from St. Louis, Missouri and graduated from Cor Jesu Academy in 2012. I graduated in May 2016 with a Bachelor of Science in Animal Science and minors in Wildlife Habitat and Environmental, Soil and Water Science. During my undergraduate career I served as the Vice-President of the University of Arkansas Wildlife Society student chapter. I have completed internships at the Saint Louis Zoo, Endangered Wolf Center and worked at the Beaver Watershed Alliance as a Microsoft Word - Long_Table 1 GAIL REV.docx.pdf Conservation Intern. In June 2016, I plan to move to Logan, Utah to pursue a master's degree in Agricultural Extension and Education at Utah State University.

I would like to thank Ken Coffey for his guidance as my academic advisor, research mentor, and professor. I would like to thank Dirk Philips and Charles Rosenkrans for providing guidance and serving on my committee. I would like to thank James Caldwell from Purina Animal Nutrition Center for providing funding and guidance. I would also like to thank Robert Rhein and Ashley Young for their guidance and assistance.

Introduction

Calcium is essential for growth and maintenance of bones and teeth and is the most abundant mineral in the body (Soares, 1995). Ninety-nine percent of the body's calcium is located in the skeleton and the remaining 1% is crucial for cellular metabolism, blood clotting, enzyme activation, and neuromuscular action (Soares, 1995). Calcium bioavailability is greatly affected by the calcium-to-phosphorus ratio in the diet (Kim et al., 1985; Albanese et al., 1986; Lopes and Perry, 1986). In high-concentrate diets, calcium addition is required to balance the excessive levels of phosphorus in the diet. The optimal calcium-to-phosphorus ratio is 2:1, but ruminants can tolerate relatively large calcium-to-phosphorus ratios if magnesium concentration in the diet is not great (Chester-Jones et al., 1990).

Calcium carbonate is commonly used as a supplemental calcium source because it is inexpensive and because of its buffering capacity. When the availability of calcium from dried skim milk was rated at 100, Greger et al. (1987) estimated the availability of calcium carbonate at 102% in comparison. Other sources of calcium are available that have potential value as supplements for which little information is available. Therefore, the objective of this study was to determine the bioavailability of calcium and magnesium in growing goats offered different calcium sources.

Materials and Methods

The University of Arkansas Animal Care and Use Committee approved all procedures (IACUC #15062). Spanish × Boer crossbred goats (n = 18; 19.6 ± 1.88 kg) were purchased from a single source, vaccinated against eight clostridial strains (Covexin 8®; Merck Animal Health, Intervet, Inc., Madison, N.J.), dewormed with levamisole hydrochloride (Prohibit® Soluble Drench Power Anthelmintic, Agri Laboratories, Ltd., St. Joseph, Mo.), and co-mingled on a predominantly bermudagrass (*Cynodon dactylon* L.) pasture. Goats were offered increasing amounts of a control, corn-based high-concentrate diet on the pasture until they were consuming the diet at 2% of body weight (BW). They were then stratified by BW and sex and allocated randomly to dietary treatments consisting of a commercially available pelleted diet containing either calcium carbonate (CC), Calmin (CM) or Milk Cal (MC). Calmin is sourced from red algae off of the coasts of Ireland and Iceland. Milk Cal is calcium sourced from milk. Diets were formulated to contain 16% crude protein (CP), 0.6% calcium, 0.48% phosphorus, 0.5% magnesium and 1.4% potassium. However, in the actual diets, calcium composition was slightly greater in CC in comparison to MC and CM (Table 1) with no difference in calcium composition between MC and CM. Magnesium and K composition was similar among the three diets.

Goats were moved to individual 1.0 × 1.5-m pens with plastic-coated expanded metal floors located in an insulated metal barn with exhaust ventilation and adjusted to their respective diets along with removal of hay from the diet over a 7-d period. Goats were then offered their respective diets at 8:30 AM and 5:00 PM for an additional 14-d adaption period to diet and facilities followed by a 7-d collection of total urine, feces and orts. Orts and fecal samples were collected at 8:30 AM daily, weighed, and dried to a constant weight at 50 °C in brown paper bags. Total urine for each goat was collected daily at 8:00 AM, weighed, mixed thoroughly, and a 10% aliquot by volume was placed in individual plastic containers and stored frozen (-20 °C). Forty mL of HCL (50% v/v) were added prior to each daily urine collection to prevent ammonia volatilization by microbial action. Urine samples were composited across days within goat.

Representative samples of feed were taken daily as feed was weighed for the goats and dried to a constant weight at 50 °C in brown paper bags. After drying, feed and fecal samples were allowed to equilibrate to atmospheric moisture in a temperature-controlled room (20 °C) and ground to pass a 1-mm screen in a Wiley mill (Author H. Thomas, Philadelphia, Pa.).

Concentrations of calcium and magnesium were determined using inductively coupled plasma emission spectroscopy after wet ashing with concentrated trace mineral grade nitric acid (ICP-OES; Method 985.01; AOAC, 2000). All laboratory analyses were corrected to a dry matter (DM) basis (Method 934.01; AOAC, 2000).

Data were analyzed using PROC MIXED of SAS with animal considered the experimental unit and treatment as the fixed effect. Sex and the sex × treatment interaction were included in the original model but the interaction was not significant ($P \geq 0.63$) for any of the variables measured. Therefore, these effects were removed from the final model. One goat on the CC diet and one goat on the MC diet did not consume their diets and their data were therefore excluded from the data analyses. Treatment means were compared using an F-protected *t*-test. All data are reported as least-squares means.

Results and Discussion

Dry matter intake (g/d) and apparent digestibility (%) did not differ ($P = 0.97$ and 0.14, respectively) among the three diets (Table 2). Calcium intake (g/d) did not differ ($P = 0.12$) among the three diets (Table 3). Calcium apparent absorption and retention (g/d and % of Ca intake) were greatest ($P < 0.05$) from CC, but did not differ ($P \geq 0.20$) between MC and CM.

Magnesium intake (g/d) did not differ ($P \geq 0.12$) among the three diets (Table 4). Magnesium apparent absorption and retention (g/d and % of Mg intake) were greatest ($P < 0.05$) from CC and did not differ ($P \geq 0.69$) between CC and MC.

In this study we compared the bioavailability of calcium carbonate, Calmin, and Milk Cal from the commercial pellet Antlermax 16. Antlermax 16 is a high-concentrate diet formulated to contain 16% crude protein and is

Table 1. Calcium, magnesium, and potassium composition of Antlermax 16 diets with different calcium sources that were offered to growing goats.

	Diets[†]		
Composition	**Calcium Carbonate**	**Calmin**	**Milk Cal**
Calcium, %	0.77	0.59	0.60
Magnesium, %	0.50	0.46	0.46
Potassium, %	1.36	1.36	1.43

[†] Calmin is calcium extracted from red algae; milk calcium is calcium extracted from milk.

Table 2. Dry matter (DM) intake and digestibility by growing goats offered Antlermax 16 with different calcium sources.[†]

	Diets[‡]			
Item	**Calcium Carbonate**	**Calmin**	**Milk Cal**	**SEM[§]**
DM intake, g/d	368	372	372	1.87
DM digestibility, %	75.5	72.2	72.9	32.97

[†] Means among treatments were not different ($P \geq 0.14$).

[‡] Calmin is calcium extracted from red algae; milk calcium is calcium extracted from milk.

[§] SEM = Standard error of the mean.

intended as a complete diet for growing deer to help support antler growth. However, goats were the experimental model instead of deer, because goats have similar digestive tracts (Van Soest, 1994) and are easier to collect fecal and urine samples from within metabolism crates than deer. Knowing the absorption and retention of calcium from Calmin, Milk Cal, and calcium carbonate is useful for determining the appropriate source and amount that should be added to the diet in order to maintain optimal horn growth and strength in deer.

Calcium source did not affect diet digestibility in the present study. Digestibility of cattle feedlot diets was not affected by substituting dolomitic limestone—a calcium source with demonstrated lower availability of calcium and magnesium—for calcium carbonate (Crawford et al., 2008). Growing pigs offered diets with calcium carbonate consumed more feed than those offered diets with a number of other calcium sources, but feed conversion efficiency was not affected by calcium source (Ross et al., 1984). In this study, diet intake was restricted, which also potentially limited differences in digestibility due to calcium source. Other data pertaining to the impacts of different sources of calcium on digestibility of high-concentrate diets for ruminants is limited.

The diets in this study were formulated to contain 0.6% calcium but the CC diet actually contained more calcium (0.77%) compared with MC (0.6%) and CM (0.59%). Although not different statistically (P = 0.12), this differential calcium concentration along with offering all diets at 2% of BW resulted in goats that were offered CC consuming 0.61 and 0.58 g/d more calcium than goats offered CM and MC, respectively. The calcium from the CC diet was the most bioavailable in comparison to MC and CM. However, this was likely not a result of the numerical increase in calcium intake because the proportion of calcium absorbed from diets is generally inversely proportional to diet calcium concentrations (Pond et al., 2005).

In a summary across a number of mammal species, calcium carbonate had equivalent Ca absorption to a number of other calcium sources including nonfat dry milk and dried skim milk (Soares, 1995). Ross et al.

Table 3. Calcium balance by goats offered Antlermax 16 with different sources of supplemental calcium.

	Diets[†]			
Item	Calcium carbonate	Calmin	Milk Cal	SEM[‡]
Intake, g/d	2.82	2.21	2.24	0.878
Apparent absorption, g/d	0.97[a]	0.27[b]	0.22[b]	0.170
Apparent absorption, %	32.4[a]	11.6[b]	10.1[b]	5.71
Retained, g/d	0.91[a]	0.24[b]	0.18[b]	0.172
Retained, % of intake	30.1[a]	10.1[b]	8.1[b]	5.85
Retained, mg/kg body wt.	43.7[a]	11.4[b]	9.2[b]	7.11

[a,b] Means within a row without a common superscript letter differ (P < 0.05).

[†] Calmin is calcium extracted from red algae; milk calcium is calcium extracted from milk.

[‡] SEM = standard error of the mean.

Table 4. Magnesium balance by goats offered Antlermax 16 with different sources of supplemental calcium

	Diets[†]			
Item	Calcium carbonate	Calmin	Milk Cal	SEM[‡]
Intake, g/d	1.85	1.71	1.71	0.155
Apparent absorption, g/d	0.93[a]	0.52[b]	0.48[b]	0.088
Apparent absorption, %	49.2[a]	31.1[b]	29.9[b]	4.00
Urine Mg, g/d	0.47	0.47	0.41	0.058
Retained, g/d	0.46[a]	0.05[b]	0.08[b]	0.073
Retained, % of intake	24.1[a]	2.9[b]	5.2[b]	4.12
Retained, mg/kg body wt.	23.1[a]	2.5[b]	4.5[b]	3.74

[a,b] Means within a row without a common superscript letter differ (P < 0.05).

[†] Calmin is calcium extracted from red algae; milk calcium is calcium extracted from milk.

[‡] SEM = standard error of the mean.

(1984) reported that growing pigs fed diets with calcium carbonate consumed more feed compared with pigs fed diets with a number of other calcium sources but calcium source did not affect other measurements including femur strength and relative calcium bioavailability. Human subjects showed no effect of calcium carbonate versus milk on urinary calcium concentrations (Martini and Wood, 2002). Therefore, calcium carbonate appears to be comparable to other more expensive calcium sources in its value as a calcium source for small ruminants.

The only supplemental magnesium source used in these diets was magnesium oxide, which is considered the standard magnesium source in livestock diets. Since calcium and magnesium can potentially affect the absorption of each other (Pond et al., 2005), it was also necessary to determine the impacts of calcium source on magnesium absorption. The CC diet had the greatest apparent absorption and retention of magnesium compared with the diets with CM and MC. Therefore, calcium carbonate does not appear to have a negative impact on magnesium bioavailability compared with other supplemental calcium sources.

Summary and Conclusions

Calcium and magnesium were more available for goats consuming the diet containing calcium carbonate compared with the diets containing Calmin and Milk Cal. Calcium carbonate is also less expensive typically than the other sources evaluated in this study. Therefore, it is not necessary to include these more expensive sources in order to improve calcium and magnesium bioavailability.

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Evaluation of harvest time/ temperature and storage temperature on postharvest incidence of red drupelet reversion development and firmness of blackberry (*Rubus* L. subgenus *Rubus* Watson)

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Abstract

Since 1964, the University of Arkansas blackberry breeding program has worked to improve fruit quality and shipping capabilities. A major limitation in blackberry fruit is postharvest handling potential for the shipping market. Maintaining fruit firmness in storage is crucial. Red drupelet reversion (or simply reversion) is also an important postharvest disorder in which drupelets change from black to red during storage. It is hypothesized that reversion is increased when fruit is picked at hot temperatures and exposed to a rapid change of temperature. These studies evaluated harvest time/temperature, as well as storage temperature, on berry firmness and the incidence of reversion. In Study One, eight genotypes were evaluated. Fruit was harvested at four harvest times (7:00 AM, 10:00 AM, 1:00 PM and 4:00 PM) and then stored for 7 d at 5 °C before evaluation. Results indicated significant sources of variation were genotype and time of harvest for the variables compression (a measure of firmness) and incidence of reversion. Breeding selection A-2453T maintained high firmness and low incidence of reversion after storage compared to other genotypes. Reversion was also significantly lower at the 7:00 AM harvest time compared to later harvests. Study Two included two genotypes harvested at 7:00 AM and 1:00 PM which were evaluated at different storage temperatures (5 and 1 °C). No significant effects were found; however, trends suggested that A-2453T maintained higher firmness despite storage temperature. These studies confirm differences in firmness and reversion among genotypes as well as reveal harvest time impact on reversion.

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Meet the Student-Author

Jack McCoy

I grew up in Little Rock, Arkansas and came to Fayetteville to attend the University in 2012. Upon arriving, I was unsure of what I wanted to study. After taking an introductory plant science course my freshman year, I decided to declare a major in Horticulture and have not looked back since. The more I learned about the horticulture sciences the more my interests grew. During my undergraduate career I have had the opportunity to work on a variety of research projects, was the program assistant for the National Strawberry Sustainability Initiative, a Teaching Assistant for Principles of Horticulture and served as the President of the Horticulture Club. In the Fall of 2014 I had the opportunity to study and conduct research in Santiago, Chile. This solidified my interest in research. I learned about the wonders of plant breeding through my academic and research mentor, John Clark and now know this is the field where I can make a significant impact. I graduated from the University of Arkansas in May of 2016 and will be pursuing a graduate degree under the direction of Paul Bosland, a world renowned chile pepper breeder at New Mexico State University.

Introduction

Blackberry (*Rubus* L. subgenus *Rubus* Watson) is an important fruit crop in the Rosaceae family. It plays an important role in both the fresh and processed market and its interest to growers and consumers has increased greatly in recent years. In the early 1990s, blackberry markets were small, localized operations found mainly in pick-your-own and local fresh markets. Poor postharvest handling attributes prevented the fruit from being shipped long distances (Clark, 2005). With significant cultivar improvements came a great increase in production from the later 1990s on. According to the Agricultural Marketing Resource Center (Geisler and Morgan, 2012), blackberry production in the United States was valued at $30.8 million in 2009 and just two years later it was estimated at $43.2 million. With expanding interests from both growers and consumers, improvements in breeding and postharvest handling are crucial.

The blackberry is a perennial plant with biennial canes where vegetative canes (primocanes) are produced in the first year and are followed by the flower/fruiting growth period (floricanes). Blackberry produces an aggregate fruit that consists of a number of drupelets, each containing a seed (pyrene), which form around the torus (Moore and Skirvin, 1990).

A major concern in fresh market blackberries is the retention of color in drupelets (Clark and Finn, 2011). Known as "red drupelet reversion" or just "reversion", blackberry often develops red drupelet color after harvest. It is thought that when fruits are exposed to a drastic change in temperature, cell organelle membranes, specifically the vacuole, break apart. The vacuole is a large organelle that can occupy 90% of a mature cell. It accumulates sugars, organic acids, aromas, flavors, ions, and water and rupturing in the membrane can cause changes in the pH of the fruit (Fontes et al., 2011). This contributes to color change (reversion) in the drupelets, resulting in an unattractive berry that is not desirable in the market. Retention of color in blackberry can be selected for, but cannot be evaluated in the field (Clark and Finn, 2011).

The University of Arkansas System Division of Agriculture's Breeding Program utilizes a standard postharvest protocol in evaluating breeding selections and cultivars for storage potential (Clark and Perkins-Veazie, 2011). The protocol evaluates berry firmness, leakage, and reversion. The program has released several cultivars with improved postharvest capabilities. This protocol is usually conducted using berries that are harvested prior to 10:00 AM. 'Natchez' is a popular cultivar, and postharvest trials in Arkansas performed well in storage, usually with low reversion observed. However, when it was grown in warmer climates such as southern Georgia, 'Natchez' fruit had high levels of reversion and required harvesting prior to mid-morning before high heat was

experienced. This highlighted a need to evaluate harvests of cultivars and advanced selections in the breeding program later in the day, when berries are exposed to higher temperatures. This could allow further confidence in identifying genotypes with greater postharvest storage potential that are harvested under less optimum conditions such as high heat.

The objectives of this study were to (1) determine the impact of time of harvest/fruit temperature at harvest on the development of red drupelet reversion and firmness of blackberry fruits during postharvest storage on various cultivars and advanced breeding selections and (2) determine the effect of postharvest storage temperature on the red drupelet reversion development and firmness on a very firm, low-reversion breeding selection compared to a standard commercial cultivar.

Materials and Methods

The studies were conducted at the University of Arkansas System Division of Agriculture's Fruit Research Station, in Clarksville, on berries harvested in June and July, 2015. Fruit for the studies was harvested from one to three 3.3-m plots with the number of plots harvested varying by genotype from replicated selection trials of advanced breeding selections and commercial standard cultivars. The plants were managed according to routine blackberry production practices, including annual dormant pruning, summer tipping of canes, trickle irrigation, fertilization, and control of spotted wing drosophila (*Drosophila suzukii* Matsumura). No fungicides were applied to the plants during the harvest season. Plants were grown on a four-wire, horizontal trellis with black plastic mulch.

For both studies, fruit temperature was measured at every harvest time across all genotypes using an infrared crop temperature meter (Spectrum Technologies Inc., Aurora, Ill.). Mean fruit temperatures averaged across all genotypes and standard deviations for the means were calculated at each harvest time in order to show the use of harvest time as an appropriate indication of fruit temperature.

Study One

Study One evaluated the impact of field temperature at harvest on firmness and the development of red drupelet reversion on blackberry fruits during postharvest storage. Shiny-black fruit free of defects of eight blackberry cultivars/selections were harvested into 0.24-L commercial plastic, vented clamshells at 7:00 AM, 10:00 AM, 1:00 PM, and 4:00 PM each day with two replicates at each harvest time. Genotypes evaluated included two breeding selections, A-2453T (crispy texture) and A-2450T, as well as the commercially available cultivars Black Magic™/APF-77, Natchez, Ouachita, Osage, Prime-Ark® 45, and Prime-Ark® Traveler. Harvest was repeated twice for each genotype. Fruit was immediately stored for 7 d in cold storage at 5 °C prior to evaluations. After storage, the fruit was evaluated for firmness and reversion.

Firmness was evaluated using an iCon Texture Analyzer (Texture Technologies Corp. Hamilton, Mass.) in Newtons (N) measuring both compression and drupelet skin penetration. For each compression measurement, 10 individual fruit were placed on a flat surface and measured using a cylindrical plane probe 7.6 cm in diameter (Fig. 1). Drupelet penetration measured the skin firmness using a probe 1 mm in diameter (Fig. 2). Three

Fig. 1. Fruit compression measurement procedure utilizing a flat surface and cylindrical plane probe of 7.6 cm in diameter.

Fig. 2. Skin drupelet penetration measurement procedure utilizing a probe of 1 mm in diameter.

drupelets were measured on each of the 10 berries evaluated. Reversion was assessed on every berry harvested. Fruit was recorded for the presence of reversion or having no reversion. Percent berries showing no reversion was used in data analysis.

Data were analyzed by analysis of variance as a split-plot design using SAS v. 9.3 (SAS Institute, Inc., Cary, N.C.). Genotype served as the whole plot, split plot was time of harvest, and clamshells were the experimental unit or replication. Least square means were separated using the least significant difference procedure.

Study Two

Study Two evaluated storage temperature on postharvest handling on the firm, crispy breeding selection A-2453T and the commercial cultivar Osage. Shiny-black fruits were harvested into 0.24-L commercial plastic, vented clamshells at 7:00 AM and 1:00 PM with two replicates at each harvest. The harvest was repeated twice for each genotype. Fruits were then divided into two groups and stored for 7 d at 1 and 5 °C. Firmness and color reversion were evaluated using the same procedures as Study One.

Data were analyzed by SAS v. 9.3 as a split-split plot design with the whole plot being the genotype, split plot harvest time, split-split plot storage temperature, and clamshell as the experimental unit.

Results and Discussion

Harvest Time and Fruit Temperature

Results of average temperature of fruits for the four harvest times confirmed the differences in harvest-time temperatures (Table 1). The earlier harvest time had a cooler fruit temperature and the temperature increased throughout the day. It is important to note the large increase in temperature from the 7:00 AM to 10:00 AM harvest time of 6.1 °C with only small temperature changes from 10:00 AM onward.

Study One

Firmness. The analysis of variance of the data indicated no significant interaction effects for any sources of variation for any firmness variables measured. Main effect of genotype was significant for compression, but not penetration (Table 2). Black Magic/APF-77 had the lowest mean compression value of 4.2 N indicating the softest-fruited genotype (Table 2), although statistically it was similar to all named cultivars except 'Prime-Ark Traveler'. The firm, crispy breeding selection A-2453T had the highest firmness compression value of 9.4 N and this value was significantly higher than all other genotypes evaluated with the exception of A-2450T, another firm but not crispy breeding selection.

Table 1. Mean fruit temperature and standard deviation for all harvests and genotypes at each harvest time.

Harvest time	Mean fruit temp (°C)	Std. dev.
7:00 AM	23.1	1.68
10:00 AM	29.2	3.45
1:00 PM	31.1	3.32
4:00 PM	31.8	2.35

Table 2. Main effect means of genotypes for fruit firmness.

Genotype	Compression (N)†	Penetration (N)†
Black Magic™/APF-77	4.2a‡	0.21
Ouachita	5.8ab	0.12
Osage	6.0ab	0.12
Natchez	6.3ab	0.11
Prime-Ark® 45	6.3ab	0.13
Prime-Ark® Traveler	6.9b	0.19
A-2450T	7.8bc	0.22
A-2453T	9.4c	0.21
P value	0.03	0.75

†Mean compression and penetration values (N = Newtons).

‡Least square means separated using least significant difference procedure.

Main effect means of harvest time were also found to be significantly different for compression, but not penetration. The 4:00 PM harvest time had the highest compression value (7.1 N) indicating firmer fruit, but it was only significantly different from the 10:00 AM harvest time (Table 3). Harvest times of 7:00 AM, 10:00 AM, and 1:00 PM were not different from each other. Firmness values fluctuated slightly throughout the day, but ultimately showed highest level at the latest harvest time.

The results for firmness were not as expected. It was anticipated that there would be an interaction of genotype and time of harvest since it had been shown in unpublished research that the genotypes varied in postharvest variables including firmness, and it was thought that the firmest selections would maintain greater firmness while softer genotypes would get softer at later harvest times that had warmer temperatures. It is not fully clear why this expected result was not seen. A possible reason is that the harvest season in 2015 was wetter than normal, as rains occurred one or more times each week during harvest, and might have reduced the potential firmness of the firmer genotypes in the study. Additionally, more replications or harvest dates could have reduced variation in the data resulting in more significant differences, although the means were not that greatly different in practical values.

It was of note that there were differences among means for compression but not penetration. A similar finding was reported by Salgado (2015). This indicates that compression is a more useful firmness measurement compared to penetration and would likely be the only measurement recommended in further investigations.

The time of harvest results were unexpected also. It was anticipated that berries would become softer as temperatures rose during the day. The opposite was found. There were no reports located in the literature that measured firmness during the day or as temperatures increased. Possibly berries became firmer due to reduced water content later in the day. However, water content was not measured in the study.

Red Drupelet Reversion. No significant interaction effects were found with incidence of reversion; however, main effect means were significant for genotype and time of harvest. Breeding selection A-2453T had very little incidence of reversion with a mean of 74.4% of fruit showing no reversion and was significantly lower than all other genotypes (Table 4). Similar to firmness evaluations,

Table 3. Main effect means of harvest time for fruit firmness averaged across all eight genotypes.

Harvest time	Compression (N)†	Penetration (N)†
7:00 AM	6.6ab‡	0.17
10:00 AM	6.0a	0.16
1:00 PM	6.6ab	0.16
4:00 PM	7.1b	0.17
P value	0.03	0.23

†Mean compression and penetration values (N = Newtons).

‡Least square means separated using least significant difference procedure.

Table 4. Main effect means of genotype for percent fruit showing no red drupelet reversion (RD_0).

Genotype	RD_0 (%)†
Black Magic™/APF-77	27.1a‡
A-2450T	40.8ab
Ouachita	43.2ab
Prime-Ark® Traveler	43.2ab
Natchez	54.2b
Prime-Ark® 45	54.6b
Osage	59.3b
A-2453T	74.4c
P value	0.01

†Percent fruit with no red drupelets.

‡Least square means separated using least significant difference procedure.

Black Magic/APF-77 was on the opposite end of the spectrum with the highest incidence of reversion with only 27.1% of berries showing no reversion. The other genotypes ranged between the two extremes, although A-2450T, 'Ouachita' and 'Prime-Ark Traveler' were statistically similar to 'Black Magic'/APF-77.

Incidence of reversion was also significant for harvest time (Table 5). The 7:00 AM harvest time had an average of fruit showing no incidence of reversion of 56.9%, significantly different than all other harvest times. An increase in reversion development can be seen at harvest times after 7:00 AM although there were no differences among other times.

The findings for reversion for main effects of genotype and harvest time were largely as expected, although it was anticipated there would be an interaction of main effects for reversion. Among genotypes, the crispy, firm A-2453T performed as expected and, as had been found in previous research (Salgado, 2015), as well as 'Black Magic'/APF-77 which had been reported to have high reversion (Clark et al., 2014). It was surprising that the firm-fruited cultivar Ouachita as well as 'Prime-Ark Traveler' were not different from 'Black Magic'/APF-77. However, environmental effects as well as number of samples and harvest dates might have impacted results, as discussed for compression.

It was anticipated that the earlier harvest time would result in lower reversion, and this was confirmed in the findings. It was also anticipated that reversion might increase with later harvest times at least for some genotypes. This was not found however, but then when one examines fruit mean temperatures for harvest time (Table 1), it is seen that fruit temperatures did not increase as much with later harvests (increase of approximately 2 °C from 10:00 AM to the later harvests) compared to 7:00 AM and 10:00 AM where a 6.1 °C increase in temperature was seen. Therefore, the findings indicate there may be a relationship between fruit temperature increase and increased reversion of blackberries. Further research is needed to confirm this result, however. The finding of increased reversion with later harvest times parallels that of the Georgia grower with 'Natchez' (J.R. Clark, pers. comm.). This finding does not fully support the idea that later harvests at higher temperatures are needed in the breeding program to evaluate a genotype's postharvest potential, since the interaction of genotype and time of harvest was not significant, indicated by parallel performance of genotypes in reversion with later harvests.

Study Two

Firmness. Analysis of variance showed no significance for either firmness measurement for main effect or interaction sources. This was surprising because prior observations of 'Osage' and A-2453T indicated the possibility of different firmness levels at harvest and in storage (J.R. Clark, unpublished data). Although no significant differences were found, there were trends in the data which suggest that A-2453T had a higher overall firmness than 'Osage' for both measurements and harvest times as well as storage temperatures (Table 6).

It was anticipated that significant differences in firmness would be observed between cultivars. Salgado (2015) found that crispy textures, such as A-2453T, were significantly higher in both compression and penetration values than their non-crispy counterparts. Study One also showed significant differences between 'Osage' and A-2453T. The trend towards A-2453T showing higher overall firmness suggests that increasing replications could increase the likelihood of finding significant differences. It is also important to note that June and July 2015 made for an unusually wet harvest season and could have affected postharvest data collection.

Red Drupelet Reversion. Incidence of reversion also showed no significant differences and no clear trends (Table 6). It is thought that if the study were repeated with a larger set of replications, significant differences could be observed for storage temperature, harvest time, and genotype.

Salgado (2015) reported significant differences in incidence of reversion between crispy and non-crispy textures. Once again, it is suspected that significant differ-

Table 5. Main effect means of harvest time for percent fruit showing no red drupelet reversion (RD_0) averaged across all eight genotypes.

Time	RD_0 (%)[†]
7:00 AM	56.9a[‡]
10:00 AM	49.1b
1:00 PM	47.4b
4:00 PM	45.2b
P value	0.001

[†]Percent fruit with no red drupelets.

[‡]Least square means separated using least significant difference procedure.

ences would likely be found with increased replications and that the unusually wet harvest season affected results. However, when comparing these results with Study One, it can be seen that 59.3% of fruit collected from 'Osage' in Study One had no incidence of reversion, second to that of the lowest genotype, A-2453T. Overall reversion values were close to this mean value for 'Osage' in Study Two, although the reversion values were significantly different for these two genotypes in Study One.

Interestingly, storage temperature played a small role in firmness and the development of reversion. Previous studies indicated that storage temperature can have a significant effect on compression and reversion, but not penetration (Salgado, 2015). It is possible that a five degree difference between storage temperatures is not large enough for observable effects.

These results do not support the idea that storage temperature and harvest time affect firmness and incidence of reversion because of lack of significance both in main effects and interaction, but trends in the data as well as results of Study One suggest a need for continued research.

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Table 6. Interaction means of storage temperature and harvest time for berry firmness and incidence of red drupelet reversion across the two genotypes. No significant differences were found in the data.

Genotype	Storage temperature	Compression (N)†	Penetration (N)†	RD_0 (%)‡
		Harvest 7:00 AM (21.0 °C)§		
Osage	1°C	4.6	0.09	64.9
	5°C	4.4	0.09	65.1
A-2453T	1°C	7.7	0.18	63.4
	5°C	8.1	0.27	65.1
		Harvest 1:00 PM (27.8 °C)§		
Osage	1°C	5.7	0.11	56.4
	5°C	4.7	0.11	55.4
A-2453T	1°C	8.8	0.23	55.7
	5°C	8.1	0.25	65.3

†Mean compression and penetration values (N = Newtons).

‡Percent berries with no reversion.

§Mean fruit temperature of all harvests at this time.

A step in the right direction: streambank restoration efforts at the Botanical Garden of the Ozarks

Dylan S. Milholen[*]*, Madison Brown*[†]*, Steven Thao*[§]*, and Lisa S. Wood*[‡]

Abstract

The Botanical Garden of the Ozarks (BGO) is a unique destination in Northwest Arkansas that draws more than 80,000 visitors a year. While the BGO manages low-input practices, run-off from pesticide application and synthetic fertilizers containing phosphorus and nitrogen are of concern to water quality, habitat, and overall ecological interactions of the BGO streambanks and adjacent Hilton Creek, which flows directly into Lake Fayetteville. One way to reduce pollution to waterbodies is through the use of riparian buffers. This project sought to establish a riparian buffer immediately adjacent to a portion of Hilton Creek in an effort to improve ecological functions and water quality. The hypothesis of this study is that the streambank restoration will increase plant abundance and diversity and improve riparian habitat quality, thus enhancing ecological functions of the Hilton Creek streambank. Pre- and post-restoration assessments were conducted to test this hypothesis. A streambank riparian habitat quality assessment was adapted from the Qualitat del Bosc de Ribera' (in English, 'Riparian Habitat Quality', (QBR)) index and species diversity values based from on-site plant species inventories were analyzed using a Shannon–Wiener Index of diversity. Overall, the pre-restoration QBR index value was calculated as 55 out of 100 and post-restoration QBR index value was calculated as 65 out of 100, suggesting an immediate improvement in riparian habitat quality. Inventoried plant species equated to a pre-restoration Shannon–Wiener Index of diversity value of 2.13, while the post-restoration Shannon–Wiener Index of diversity equaled 2.91, indicating an increase in species diversity. Water quality parameters were recorded to establish baseline values for Hilton Creek to encourage future monitoring of the project site as the streambank restoration matures.

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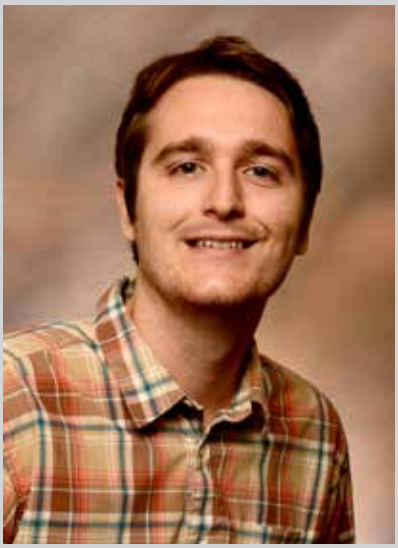
[‡] Lisa. S. Wood, the faculty mentor, is a Clinical Assistant Professor in the Department of Crop, Soil, and Environmental Science.

Meet the Student-Authors



Dylan Milholen

I grew up in Hot Springs, Arkansas and graduated from Lakeside High School in 2011. I have since graduated from the University of Arkansas in May 2016 with a B.S. in Environmental, Soil and Water Science, and a minor in Sustainabilty. During the summer of 2015, I received a Bumpers College travel award to conduct research in Pelotas, Brazil at Universidade Federal de Pelotas in an analytical biochemistry lab. I served as the Dale Bumpers College Ambassador for the Department of Crop, Soil, and Environmental Sciences, 2015-2016. I am highly interested in the transport and the transformation of chemicals and pollutants in the environment and their interactions in soil and water interfaces. I am thankful for guidance from Lisa S. Wood throughout my academic career and with this project. I am indebted to the several other professors I have worked with and learned from throughout my academic career, but a special thank you is owed to David Miller, Nilda Burgos, Esten Mason, all University of Arkansas Professors and Fabio Chaves, UFPel Professor, for their central facilitation in my intellectual growth as a student.

I was born in Dallas, Texas and graduated with honors from Plano West Senior High School. I began my time as an Environmental, Soil, and Water Science student at the University of Arkansas in the fall of 2013. I am currently the president of the Undergraduate Crop, Soil, and Environmental Science (CSES) club as well as a member of the Bumpers College Honors Program. In the summer of 2015 I participated in a four-week ecology program in Edinburgh, Scotland that allowed me to gain experience in field research and environmental policy. It was during this time abroad that I decided to pursue a career in environmental law. I have always felt passionately about the environment and I am thankful that the University of Arkansas has given me the opportunity to gain the knowledge and skills necessary for me to be a steward of this earth. I thank Dr. Wood for all of her support and guidance throughout this project. Dr. Wood has been an immensely wonderful presence throughout my undergraduate years as she has also served as my honors mentor, professor, and CSES club advisor.

Madison Brown

Introduction

The Botanical Garden of the Ozarks (BGO) is a unique destination in Northwest Arkansas that draws more than 80,000 visitors a year (BGO, 2015). According to BGO, the site includes over 40 acres with 12 themed gardens and borders Hilton Creek, which flows directly into Lake Fayetteville. While the BGO manages low-input practices by applying as little fertilizers and pesticides as possible to sustain healthy plant growth, run-off from pesticide application and synthetic fertilizers containing phosphorus and nitrogen are of concern to water quality, habitat, and overall ecology of the site. Excess nitrogen and phosphorus from fertilizers, and pollutants from pesticides frequently bond to soil particles that are deposited in nearby waterbodies from surface runoff (Hawes and Smith, 2005). One way to reduce pollution to waterbodies is through the use of riparian buffers (Cunningham et al., 2009).

A streambank restoration consisting of multiple vegetative species was designed to implement a functioning riparian buffer at the BGO. Riparian vegetation slows sediment-rich runoff and, depending upon buffer width and vegetative complexity, may absorb 50% to 100% of sediments as well as the nutrients and pollutants attached to them (CRJC, 2005). The literature suggests that fairly narrow riparian buffers (i.e., <30 m) can adequately provide multiple ecological functions (USACE, 1991). Ecological functions such as promotion of aquatic life, stream temperature control, and terrestrial wildlife habitat from vegetative diversity are central benefits of riparian buffers (Wenger, 1999).

This project sought to establish a riparian buffer immediately adjacent to a portion of Hilton Creek in an effort to improve ecological functions and water quality. The hypothesis of this study is that the streambank restoration will increase plant abundance and diversity and improve riparian habitat quality, thus enhancing ecological functions of the Hilton Creek streambank. The Qualitat del Bosc de Ribera' (QBR) index (in English, 'Riparian Habitat Quality') serves the purpose of providing a simple method to evaluate riparian habitat quality (Munné et al., 2003). A larger QBR index indicates greater riparian habitat quality. It is necessary to catalog streambank vegetative species pre- and post-restoration to test the hypothesis that the streambank restoration will increase plant abundance and diversity of the site. The Shannon–Wiener Index of diversity is a widely used index for comparing species diversity between habitats (Clarke and Warwick, 2001). A greater Shannon–Wiener Index of diversity indicates greater species diversity.

The objectives for this project were 1) to assess the streambank riparian habitat quality by comparing a pre-assessment QBR index (Munné et al., 2003) and a post-assessment QBR index, 2) catalog streambank vegetative species diversity using a Shannon–Wiener Index of diversity (Krebs, 1989), and 3) to measure baseline water quality parameters including temperature, dissolved oxygen, specific conductance, and pH for Hilton Creek adjacent to the streambank restoration site. It is essential to assess streambank riparian habitat quality in order to test the hypothesis that the streambank restoration will improve riparian habitat quality.

Materials and Methods

Initial Assessment

The streambank restoration area was divided into three zones perpendicular to the stream: Zone A (1.3 wide × 10.7-m long, variable slope of 0-2%) was located at the top of the streambank; Zone B (2.7 wide × 10.7-m long, variable slope of 40-45%) was located along the steep sideslope of the streambank; and Zone C (1.3 wide × 10.7-m long, variable slope of 0-2%) was located immediately adjacent to the stream. LaMotte soil test kits Code 5930-01 and Code 5931-01 (LaMotte Company® STH, USA) were used to measure total nitrogen and phosphorus levels, respectively. Soil pH was measured using the LaMotte kit, Code 5935-01. Measurements were taken from a discreet soil sample collected prior to restoration to better understand the soils present at the riparian site and any factors limiting vegetative growth. Two grams of soil collected with the test kit spoon from the top 10 cm of soil at the geographical center of Zone C was used as the discreet sample.

The QBR Index

The streambank riparian habitat quality assessment was adapted from the QBR index (Munné et al., 2003). A QBR index comparison was conducted pre- and post-restoration (18 March and 16 April 2016). The QBR index is based on four components of riparian habitat: 1) total riparian vegetation cover, 2) cover structure, 3) cover quality and 4) channel alterations, each given a score from 0 to 25 which are added to give a total score that varies between 0% and 100% potentially assigned corresponding to total percent riparian habitat quality.

A line-transect sampling method adapted from Thomas et al. (2002) was used to calculate total percent riparian vegetation cover and cover structure. For the line-transect sampling method used for components 1 and 2, a measuring tape was placed parallel to Hilton Creek running along the middle of each zone (A, B, and C). At each 0.107 m it was noted if vegetation touched the measuring tape (touching) or not (not touching). Equation 1 was used to find the percent cover for zones A, B,

and C which were then averaged to show total riparian vegetation cover and total tree cover.

$$\% \text{ cover} = \left(\frac{\text{touching}}{\text{total marks}}\right) * 100 \qquad \text{Eq. (1)}$$

The total riparian vegetation cover was scored: 25 if >80% of vegetative riparian cover was present at the riparian site, 10 if 50–80% of riparian cover was present, 5 if 10–50% riparian cover was present, and 0 if <10% of riparian cover was present at the riparian site. Cover structure was scored: 25 if >75% tree cover was present, 10 if 50–75% tree cover or 25–50% tree cover with 25% of riparian area covered by shrubs, 5 if tree cover was lower than 50% but shrub cover was at least between 10% and 25%, and 0 if <10% of either tree or shrub cover was present at the riparian site. Again, the percentage cover was totaled from only tree and shrub vegetation touching the measuring tape as previously described.

Components 3 and 4 were calculated based on visual site appearance and matched with the corresponding four scores (0, 5, 10, and 25). For component 3, the size of the riparian area was first noted based on its closest compatibility with three given selection types listed in Munné et al. (2003)—type 1, type 2, and type 3. Type 1 is described as a small riparian habitat (i.e., 25-900 m^2). Type 2 is described as a mid-range riparian habitat (i.e. 901-3600 m^2) and Type 3 is described as a large riparian habitat (i.e., >3600 m^2). After selecting the closest corresponding type of the riparian area, the cover quality (component 3) was scored. Cover quality was scored: 25 if the number of native tree species for type 1 was >1, for type 2 was >2, and for type 3 was >3; 10 if the number of native tree species for type 1 was = 1, for type 2 was = 2, and type 3 was = 3; 5 if the number of native tree species for type 1 was = 0, for type 2 was = 1, and type 3 was = 1 to 2; and 0 if there was an absence of native trees at the riparian site for all types

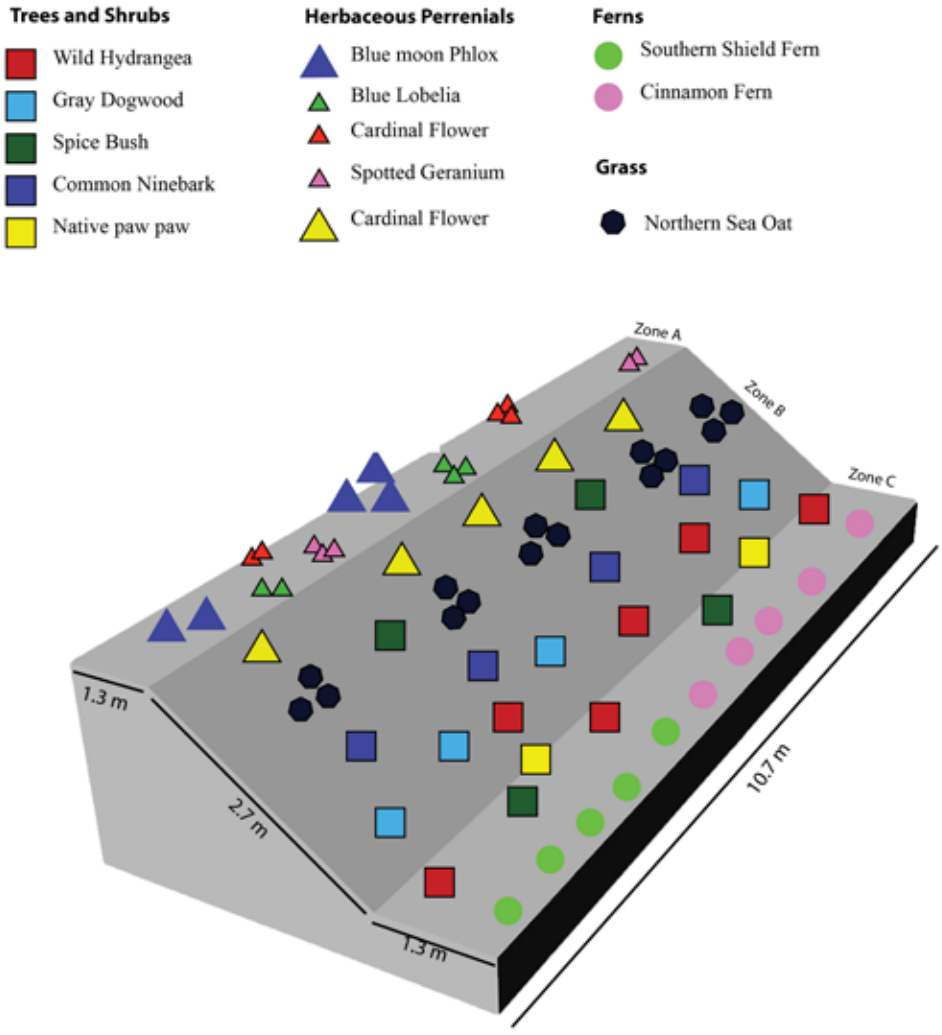


Fig. 1. Three-dimensional streambank restoration planting design using Rhinoceros® 5 and Adobe® Illustrator.

(1, 2, and 3). Channel alterations (component 4) were scored: 25 if an unmodified river channel existed, 10 if fluvial terraces were modified and constraining the river channel, 5 if the channel was modified by rigid structures along the margins, and 0 if it was a channelized stream. After scoring each of the four components, scores are added together to get a total score out of 100.

The Shannon–Wiener Index of Diversity

On-site plant species inventories were recorded (18 March and 5 April 2016) with the help of the BGO staff horticulturist. From these inventories, Shannon–Wiener Indices of diversity were calculated. The Shannon–Wiener Index of diversity (Eq. 2) represents the plant species diversity within the streambank restoration area. The index was calculated by determining the proportion each species contributes to the total population. If *S* is the total number of species in the sample, diversity is:

$$H = -\sum_{n=1}^{s} (P_i)\left|\ln P_i\right| \qquad \text{Eq. (2)}$$

where the summation sign Σ indicates that the product ($P_i \ln P_i$) is calculated for each species in turn and these products are summed together. The P_i is the proportion of individuals of each species relative to the number of individuals in the whole population. The number of plant species, *S*, and number of individuals per species were recorded 18 March and 5 April 2016 for the entire streambank restoration area (irrespective of zones) and quantified compared to the total individuals for the entire streambank restoration area, P_i. Native plant species purchased from White River Nursery and obtained from the BGO property were planted on 5 April 2016 in accordance with the design shown on Fig. 1. The recent plantings were included in the 5 April plant species inventory.

Baseline Water Quality Parameters

The methods used to measure water quality parameters in Hilton Creek, adjacent to the restoration site were adapted from the Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers (Barbour et al., 1999). In-stream measurements of dissolved oxygen, pH, specific conductance, and temperature (°C) were taken three times during the project, (7 Feb. 18 March and 16 April 2016) using a Sonde (YSI 600XLM®, USA) in accordance with Form 1: Physical Characterization/Water Quality Field Data Sheet, from APPENDIX A-1: Habitat Assessment and Physicochemical Characterization Field Data Sheet A-6 (Barbour et al., 1999). Each of the measurements was taken in replicates of three at three locations within Hilton Creek, representative of upstream (35 m upstream of the center of Zone C), midstream (center of Zone C), and downstream (35 m downstream of the center of Zone C) locations and then averaged. One-way analysis of variance (ANOVA) was run to analyze main effects of sampling time averaged across locations and location averaging the values across sampling time using JMP® Pro.

Fig. 2. Hilton Creek streambank at the Botanical Gardens of the Ozarks, Fayetteville, Arkansas shown pre-restoration, 7 Feb. 2016.

Results and Discussion

Initial Assessment

The Hilton Creek streambank pre-restoration site was devoid of vegetation with exposed soil, susceptible to erosion (Fig. 2). Post-restoration holds a diversity of native vegetation providing significant ground cover to minimize erosion (Fig. 3). Soil test results from one discreet sample from the geographical center of Zone C indicated the soil contained approximately 81.82 and 84.07 (kg/ha) of nitrate and phosphorus respectively, and had a pH of 6.9. Soil test values confirmed the site was suitable for planting desired native trees, shrubs, herbaceous perennials, ferns, and grasses arranged in the design (Fig. 1).

The QBR Index

Pre-restoration QBR index value for component 1) total riparian cover was calculated as 14% riparian cover resulting in 5 points out of a possible 25. Pre-restoration QBR index value for component 2) cover structure was calculated as 2% tree and 1% shrub cover resulting in 0 points out of a possible 25. Pre-restoration QBR index value for component 3) cover quality was scored 25 points out of 25 possible as greater than one native tree species (n = 2) was present, after matching the streambank restoration area to a type 1. Pre-restoration QBR index value for component 4) channel alteration was scored 25 points out of 25 since the stream channel boundaries appeared unmodified by human alterations.

Post-restoration QBR index value for component 1) was calculated as 64% riparian cover granting 10 points. Post-restoration QBR index value for component 2) was scored 5 points as calculations of tree cover equaled 2% and shrub cover equaled 14%. Post-restoration QBR index value for component 3) was scored 25 points, as greater than one native tree species (n = 2) of various types were present. Post-restoration QBR index value for component 4) was scored 25 points, as the stream channel boundaries remain unmodified by human alterations. Overall, the pre-restoration QBR index value was calculated as 55 out of 100 and post-restoration QBR index value was calculated as 65 out of 100, suggesting an immediate improvement in riparian habitat quality simply as a result of planting additional native species.

The Shannon–Wiener Index of Diversity

Inventoried plant species pre-restoration held a value of 10 total species and 21 total individuals from those species, while post-restoration plant species held a value of 22 total species and 94 total individuals from those species (Table 1). An addition of 12 new species and 73 individual plants from those species equated to an increased Shannon–Wiener Index of diversity value post-restoration. The pre-restoration Shannon–Wiener Index of diversity value equaled 2.13, while the post-restoration Shannon–Wiener Index of diversity equaled 2.91, indicating an increase in species diversity. Results show quantitative differences post-restoration through increased plant diversity and abundance, as well as improved riparian habitat quality.

Fig. 3. Hilton Creek streambank at the Botanical Gardens of the Ozarks, Fayetteville, Arkansas shown 5 April 2016 post-restoration, after the 3 April 2016 planting day.

Baseline Water Quality Parameters

Water temperature in the Hilton Creek in April was statistically greater than March and February, which were similar, ($P < 0.0001$; Fig. 4). Temperature averaged over time showed no statistical difference among stream sampling locations, ranging from 10.4 °C for upstream values to 11.7 °C for downstream values ($P = 0.6067$, data not shown). Dissolved oxygen declined in April compared to February and March, which were similar in concentration ($P = 0.0315$; Fig. 5). Dissolved oxygen concentration averaged over time showed no statistical difference between midstream and downstream sampling locations ranging from 9.2 mg/L downstream to 9.6 mg/L midstream, although it was statistically greater upstream from the restoration site at 11.3 mg/L ($P = 0.0007$; data not shown). Specific conductance varied over time and location with sampling dates all being statistically different ranging from 209.9 µS/cm to 369.1 µS/cm, February to April ($P < 0.0001$) and location showing no statistical difference ranging from 263.8 µS/cm to 318.8 µS/cm, upstream to downstream ($P = 0.2755$). The pH levels varied over time and location with sampling dates all being statistically different averaged at 7.1 in February, 7.9 in March, and 6.3 in April ($P < 0.0001$). The pH levels upstream averaged at 7.5 and downstream averaged at 6.7 were statistically different from each other although both were not statistically different from midstream values averaged at 7.1 ($P = 0.0884$).

Discussion

Results of increased species diversity and QBR index value indicate the streambank restoration improved riparian habitat quality, thus enhancing ecological functions of the Hilton Creek streambank. Recent experiments have provided evidence of the functional importance of biodiversity to ecosystem processes and properties (Giller et al., 2004). A study by Zedler (2000) showed that more species-rich areas achieved greater canopy complexity; thus, diversity enhanced the potential for wildlife support. Two particular plant species added in this project for wildlife support were the *Lindera benzoin* and the *Hydrangea arborescens*, commonly referred to as the Spicebush and the Wild hydrangea, respectively. The Spicebush is regarded as a sanctuary for caterpillars and the Wild Hydrangea commonly supports pollinators (Couto and Averill, 2016; Hayden, 2006). While restoration is important to restore lost biodiversity, it also provides functional landscape services, such as flood-peak reduction and water quality improvement (Zedler, 2000).

Water quality parameters were recorded to establish baseline values for the site, encouraging future monitoring of the project site as the streambank restoration matures. Dissolved oxygen is expected to be inversely related to temperature (Behar, 1997; Manasrah et al., 2006; USGS, 2016). As temperature increased in the stream in April, dissolved oxygen levels decreased in part because oxygen is less soluble in warm water than in cool water.

Table 1. Pre- and post-restoration plant species inventory recorded 18 March and 5 April 2016.

Pre-restoration[a] existing vegetation	Scientific name	Number of individuals[b]	Post-restoration[c] added vegetation	Scientific name	Number of individuals[d]
Sedge grass	*Carex spp.*	5	Northern sea oat	*Chasmanthium latifolium*	15
Chick weed	*Stellaria media*	3	Wild hydrangea	*Hydrangea arborescens*	6
Native paw paw	*Asimina triloba*	3	Cardinal flower	*Lobelia cardinalis*	8
Wild violet	*Viola spp.*	3	Blue lobelia	*Lobelia siphilitica*	5
Wild carrot	*Daucus carota*	2	Blue phlox	*Phlox divaricata*	5
Elderberry	*Sambucus spp.*	1	Red columbine	*Aquilegia Canadensis*	5
Horn beam	*Carpinus spp.*	1	Spotted geranium	*Geranium maculatum*	5
Red buckeye	*Aesculus pavia*	1	Southern shield fern	*Dryopteris ludoviciana*	5
Silky dogwood	*Cornus amomum*	1	Cinnamon fern	*Osmunda cinnamomea*	5
Wild onion	*Allium spp.*	1	Common ninebark	*Physocarpus opulifolius*	4
			Gray dogwood	*Cornus foemina*	4
			Spicebush	*Lindera benzoin*	4
			Native paw paw	*Asimina triloba*	2

[a] Pre-restoration: Species Total = 10. Note: All pre-restoration species were found again in the April assessment.
[b] Total Individuals = 21.
[c] Post-restoration: Species Total = 22.
[d] Total Individuals = 94.

Dissolved oxygen values from this study were compared to findings from Behar (1997) which suggested that even the lowest collected value of 8.9 mg/L in April was safe for most stream fish. Decreases in dissolved oxygen are important to monitor because dissolved oxygen is the oxygen that aquatic organisms use for respiration, and if it drops too low then aquatic organisms can suffocate. Freshwater streams ideally should have a conductivity between 150 to 500 µS/cm to support diverse aquatic life (Behar, 1997). Overall specific conductance measurements from the months of February to April were at desirable levels (150 to 500 µS/cm) to support aquatic life. The pH levels of the stream also varied between desirable levels from 6.1 to 8.3. It is recommended that baseline values for water quality parameters continue to be measured to better understand Hilton Creek and its interactions with the BGO streambank areas. One of the difficulties of restoration projects is the lack of baseline and reference data. This project allows others to know to what degree restoration has altered form and function by providing starting data.

Continued monitoring and adaptive management of this restoration site will play a crucial role in its overall long-term success. Adaptive management is the integration of design, management, and monitoring to systematically test assumptions in order to adapt and learn (Salafsky et al. 2001). It is assumed that a continued increase in plant species diversity will follow post-restoration, but adaptive management and continued monitoring are necessary to test and verify that assumption. Multiple studies show the importance of long-term monitoring in restoration projects and ecological studies (e.g., Franklin, 1989; Klein et al., 2007). Franklin (1989) shows that many ecological processes (i.e., plant succession, vegetative development, soil formation, biogeochemical interactions) take place over relatively long periods of time compared to grant funding time periods, making it hard to adequately account for the impact of a project with out long-term monitoring. Initial results of Klein et al. (2007), a study over a long-term monitoring program for the Lower Red River Meadow Restoration Project in north-central Idaho, U.S.A., has observed ecosystem im-

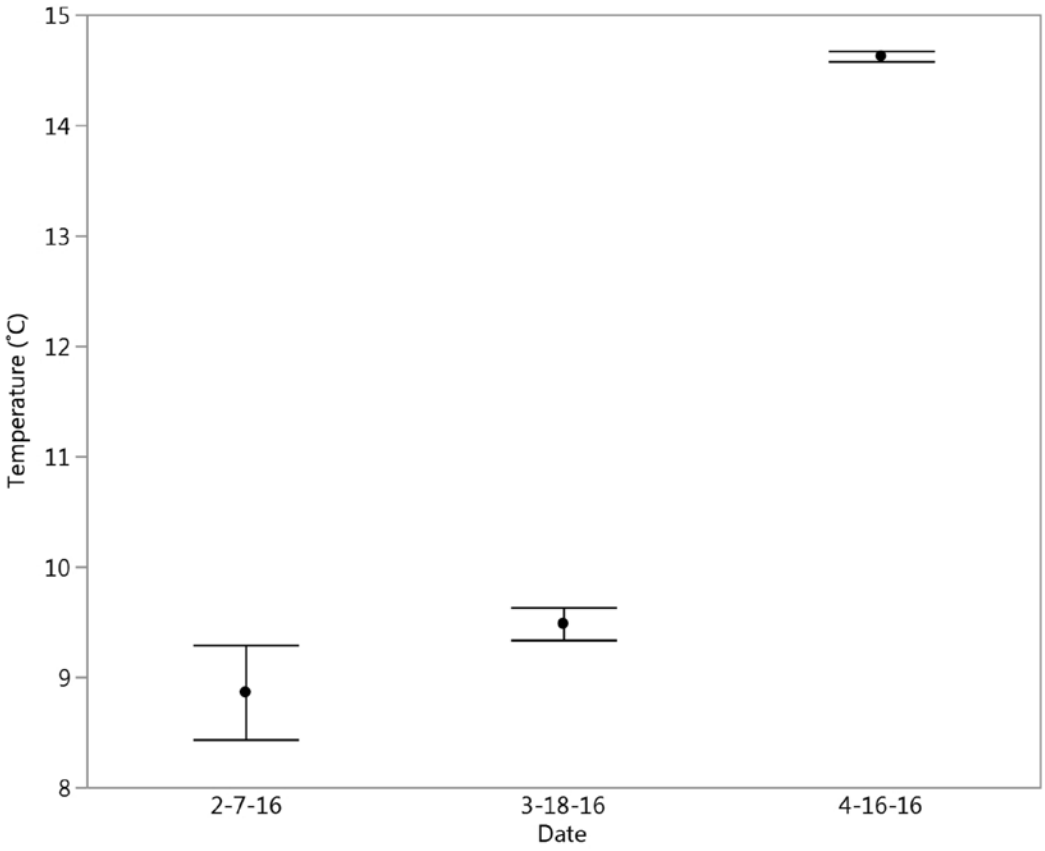


Fig. 4. Temperature (˚C) measured at three different dates, averaged across three locations adjacent to the restoration site along the Hilton Creek streambank at the Botanical Gardens of the Ozarks, Fayetteville, Arkansas, 2016. Error bars represent standard error of the mean (n = 3).

provements compared to pre-restoration conditions in channel sinuosity, slope, depth, and water surface elevation; quantity, quality, and diversity of in-stream habitat and spawning substrate; and bird population numbers and diversity. This project has provided a foundation for future study of Hilton Creek and streambanks of the BGO. An early May site visit appeared to show an even greater increase in plant species diversity and it is recommended that another species inventory be conducted during the summer months to account for vegetation that had not germinated prior to the early April inventory. There are perhaps greater benefits to be accrued from the restoration through time as later assessments could capture full germination and establishment in the restored area. Numerous studies support this hypothesis by showing increases in plant diversity from restoration efforts (Bullock et al., 2011; Le et al., 2012; Parkes et al., 2012; Rey-Benayas et al., 2009). The April assessment was limited in that there were only two and a half weeks between the time of planting and the final inventory.

A secondary outcome of this study is the opportunity for the BGO to use the site as an educational tool to teach visitors of the importance of riparian buffers and restoration efforts. In an effort to showcase the project and increase awareness, an educational sign is to be displayed near the riparian zone that recognizes some of the benefits of riparian zones. With over 80,000 visitors to the BGO annually, this project has the continued potential to educate a much broader community.

Conclusions

The BGO is a popular destination in the Northwest Arkansas community. This project demonstrated improvement in a section of the Hilton Creek streambank at the BGO and provided a foundation for further study of the site. Baseline water quality assessments of dissolved oxygen, temperature, specific conductance, and pH suggest that the Hilton Creek stream is suitable for aquatic life. Restoration efforts successfully added various veg-

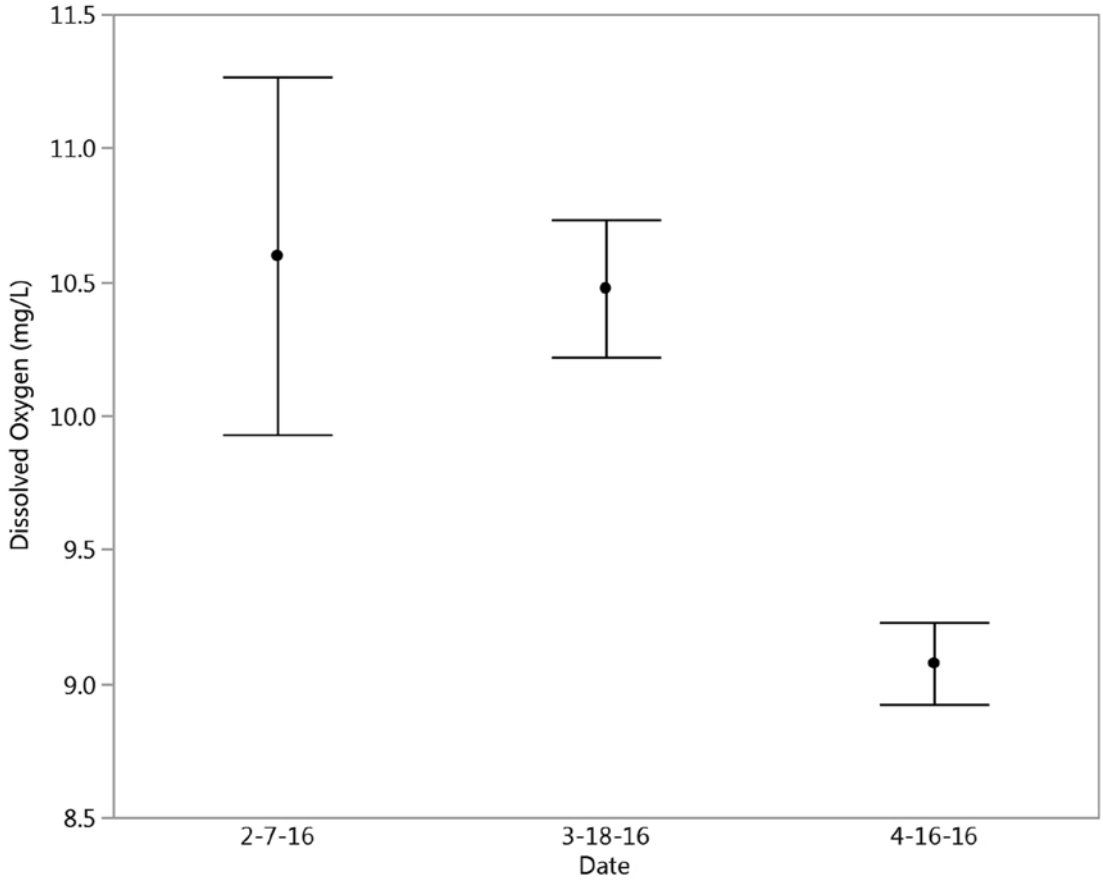


Fig. 5. Dissolved oxygen (mg/L) measured at three different dates and averaged across three locations adjacent to the restoration site along the Hilton Creek streambank, 2016. Error bars represent standard error of the mean (n = 3).

etative plant species to the streambank, supporting the hypothesis that restoration will increase plant abundance and diversity and improve riparian habitat quality, thus enhancing ecological functions of the Hilton Creek streambank. While long-term assessment is recommended to gauge the full extent of the benefits resulting from the restoration, progressing germination is expected to yield improved results. The restoration project will not only benefit the Hilton Creek streambank, but will also provide a platform to educate the BGO visitors on the significance of the chosen vegetation, riparian buffers, and restoration efforts.

Acknowledgments

The authors are thankful for funding from the Bumpers College Undergraduate Research Grant and White River Nursery. Support also provided by the University of Arkansas System Division of Agriculture. The authors also thank the Botanical Garden of the Ozarks staff for their support in this project. Acknowledgment is owed to Dallas Myers of the Fay Jones School of Architecture for the help with Rhinoceros® 5 and Adobe® Illustrator design renderings.

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Use of anti-mullerian hormone to select for fertility in beef heifers

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Abstract

A study was conducted to determine whether concentration of serum Anti-Mullerian Hormone (AMH) at weaning and/or breeding could predict subsequent fertility in beef heifers. Frequency distribution was used to assign serum AMH concentration measured at weaning, breeding, and the change from weaning to breeding into quartiles. Comparison of heifers based on serum AMH quartiles at weaning failed ($P \geq 0.35$) to detect any effect of AMH on subsequent heifer cyclicity at breeding, estrous response after synchronization, artificial insemination (AI) pregnancy rate, overall breeding season pregnancy rate, or estimated estrous cycle of the breeding season when conception occurred. Based on AMH concentration at breeding, heifers in the lowest quartile (Q1) had a lower ($P = 0.02$) AI pregnancy rate than heifers in other quartiles, and conceived at a later estrous cycle ($P = 0.03$) in the breeding season. Comparison of heifers based on the difference between AMH concentrations at breeding versus weaning revealed that none of the heifers in the lowest quartile (Q1) became pregnant after AI, compared with 80% in the highest quartile (Q4; $P < 0.001$). Heifers in the lowest quartile also conceived at a later estrous cycle in the breeding season than heifers in the other quartiles ($P = 0.01$). Results indicate that either AMH concentration at breeding or the change in AMH from weaning to breeding can identify beef heifers more likely to conceive to AI and to conceive early in the breeding season.

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Meet the Student-Author

Hannah Newberry

I was raised in Harrison, Arkansas where I graduated as valedictorian from Harrison High School in 2012. I chose to continue my education at the University of Arkansas, graduating summa cum laude in May 2016 with a major in Animal Science with the Pre-Veterinary concentration. Throughout my undergraduate career, I was a member of the Pre-Vet club for three years which helped me gain useful insight in order to apply for vet school in the summer of 2015. I have also been working part time as a veterinary assistant at the Harrison Animal Clinic for the past two years. In April 2016 I was accepted to the Oklahoma State University College of Veterinary Medicine Class of 2020. My future plans include pursuing a career in both large and small animal medicine.

I would like to thank my mentor Rick Rorie for all of his guidance and making this research project possible. I truly appreciate all of the time and effort he put into helping me. In addition, I would also like to thank my other two committee members, Beth Kegley and Charles Rosenkrans for their support for my thesis project and many other aspects of my undergraduate experience. Recognition should also be given to Toby Lester, who completed the ultrasonography and blood sampling, and Mohan Acharya and Chris Hansen who assisted with the anti-Mullerian Hormone assays for the project.

Introduction

The number of follicles present in the ovaries of heifers at birth range from 10,000 to 350,000 (Erickson, 1966). Heifers with low follicle counts also have smaller ovaries and fewer morphologically healthy follicles and oocytes, suggesting a link between follicle number and fertility (Ireland et al., 2008). Anti-Mullerian Hormone (AMH) is produced by granulosa cells of various size follicles (up to a size of 4 to 5 mm diameter), and reflects the total number of healthy follicles within the ovaries (Visser et al., 2006). Therefore, the measurement of AMH in circulation might be used as an indicator of fertility.

Anti-Mullerian Hormone is detectable as early as 36 weeks gestation in the ovarian follicles of developing heifer calves (Rajpert-De Meyts et al., 1999). A single measure of AMH in the circulation of breeding age heifers has been used to identify heifers with greater reproductive potential (Ireland et al., 2011). The question arises as to how early in development AMH can be measured as an indicator of fertility. Identification of heifers with low or high fertility at birth or weaning would be advantageous to producers for making management decisions. Therefore, the objective of the present study was to examine the relationship between serum AMH concentration at weaning versus breeding, and to determine if either or both measures could predict subsequent fertility of beef heifers.

Materials and Methods

Animal Management

The study utilized 71 beef heifers located at the University of Arkansas System Division of Agriculture's Beef Research Unit near Savoy, Arkansas. Prior to the study, all proposed animal procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (IACUC protocol # 15041). At weaning (~7 months of age), a 10-mL blood sample was collected in serum separator tubes, labeled, and the serum was frozen (-20 °C) until analysis for Anti-Mullerian Hormone (AMH). The heifers were then developed and maintained on pasture, with access to free-choice mineral, and provided corn gluten feed to meet energy requirements as needed. At breeding age (~14 months of age), a second 10-mL blood sample was collected in serum separator tubes, serum recovered and frozen.

Approximately 30 days before the start of the breeding season, transrectal ultrasonography (IBEX Pro with a L6.1 linear array transducer; E.I. Medical Imaging, Loveland, Colo.) was performed to determine the reproductive tract score (Anderson et al., 1991) of each heifer. At the time of reproductive tract scoring, a scan through the left and right ovary of each heifer was video recorded in order to accurately determine ovary size, the presence or absence of a corpus luteum, and the number and size of the larg-

est follicles present. Based on ovary size and structures present, heifers were categorized as cyclic or non-cyclic.

Estrous Synchronization and Breeding

At the start of the breeding season all heifers received a single 25-mg intramuscular (i.m.) injection of prostaglandin F2alpha (Lutalyse; Zoetis, Florham Park, N.J.) and an estrous detection patch (Estrotect; Rockway Inc., Spring Valley, Wis.). Heifers were observed 3 or more times daily for onset of estrus, and inseminated approximately 12 hours after detected estrus. Heifers not detected in estrus received a second Lutalyse i.m. injection 7 days after the initial treatment. Estrus detection and insemination continued for 4 days as previously described. Ten days later, the heifers were exposed to fertile bulls for a 45-day breeding season. Bulls were rotated through breeding groups half way through the breeding season. At 50 to 60 days after insemination, transrectal ultrasonography was used to identify pregnant heifers and to confirm conception date, based on fetal crown-to-rump length. At 60 days after bull removal, transrectal ultrasonography was used again to determine pregnancy in heifers conceiving during the breeding season and confirmed a continuing pregnancy in heifers previously identified as pregnant. Based on fetal size at ultrasonography, the estrous cycle after initiation of breeding when conception occurred was estimated. For comparison, artificially inseminated (AI) pregnancies were considered cycle 0, and pregnancies initiated during the first, second or third 21-day intervals of the breeding season were classified as cycles 1, 2 and 3, respectively.

Anti-Mullerian Hormone Assay

Serum samples were analyzed for AMH, using bovine AMH ELISA kits (Ansh Labs, Texas), and following procedures as outlined by the kit. Each assay plate contained a standard curve in duplicate, ranging from 0 to 2.4 ng/mL AMH. Two kits were utilized: one for the serum samples collected at breeding and the other at weaning. The ELISA kit was a 3-step sandwich type immunoassay using 96-well plates, with each well coated with biotinylated AMH antibody. Standards, high and low controls, and unknowns (50 µl) were added to appropriate wells, along with 50 µl of assay buffer. Each assay plate was then incubated 2 hours on an orbital plate shaker (Titer Plate Shaker, Lab-Line Instruments, Melrose, Ill.) at room temperature. Plates were then washed 5 times, using an automated plate washer (ELP-40 Microplate Strip Washer, Bio-Tek Instruments, Winooski, Vt.).

An AMH antibody-biotin conjugate (100 µl) was added to each well, followed by another incubation on the plate shaker for 1 hour. After washing 5× again, 100 µl of streptavidin-enzyme conjugate was added to each well, followed by incubation on the plate shaker for 30 minutes. Following another 5× plate wash, 100 µl of Tetramethylbenzidine (TMB) chromogen was added to each well and the plates placed back on the plate shaker. Visual color change was monitored and after 12 minutes, the plate was removed and 100 µl of stopping solution was added to each well to prevent further color change. Within 15 minutes of addition of stopping solution, the plates were read (0.1 second/well) for absorbance at 450 nm, using a Perkin-Elmer (Waltham, Mass.) Victor V, Model 1420 Multi-label Counter. Absorbance readings for 'blank" wells were subtracted from all other well readings to correct for plate optical density.

Statistical Analysis

All data analysis was performed using JMP Pro 12.0 statistical software (SAS Institute, Inc., Cary, N.C.). Regression analysis (bivariate fit) was used to determine the relationship between absorbance readings and standard concentrations of AMH. The resulting regression equations were used to calculate AMH concentration in each unknown sample within the appropriate assay plate. Frequency distribution was also used to assign AMH concentration measured in serum samples at weaning and breeding to quartiles. In addition, quartiles were established for the difference or change in AMH from weaning to breeding (breeding-weaning AMH). Comparisons were then made for heifers in each quartile and the percentage of heifers cyclic at synchronization, expressing estrus after synchronization, conceiving after artificial insemination, pregnant at the end of the breeding season, and the estimated cycle after the initiation of breeding that conception occurred.

Results and Discussion

The heifers weighed an average of 240.6 ± 2.5 kg at weaning and 358.6 ± 3.7 kg at the start of the breeding season. Transrectal ultrasonography determined that 39/71 (55.0%) of the heifers were cyclic before the start of breeding. After 2 (7 days apart) injections of prostaglandin F2alpha to induce estrus, 48/71 (67.6%) of heifers were detected in estrus and inseminated. Twenty-two of forty-eight (45.8%) of the heifers conceived after artificial insemination. At ultrasonography ~60 days after the breeding season 62/71 (87.3%) of the heifers were confirmed to be pregnant.

The inter-assay variation among duplicate samples in the AMH assay performed for serum samples collected at weaning was 2.7%. The regression equation (R^2 = 0.998) used to determine AMH concentration in serum collected at weaning was: AMH ng/mL = -0.034853 + 0.5789461*absorbance. At weaning, serum AMH

ranged from 0.04 to 0.99 ng/mL, with a mean of 0.30 ng/mL. The inter-assay variation among duplicate samples in the AMH assay performed for serum samples collected at breeding was 3%. The regression equation (R^2 = 0.995) used to determine AMH concentration in serum collected at breeding was: AMH ng/mL = -0.080924 + 0.577811*absorbance. At breeding, serum AMH ranged from 0.04 to 1.73 ng/mL, with a mean of 0.56 ng/mL.

When heifers were compared by quartiles, based on serum AMH at weaning, AMH hormone concentration at that time had no effect ($P \geq 0.35$) on subsequent heifer cyclicity at breeding, response to synchronization, AI pregnancy rate, overall pregnancy rate, or mean cycle of the breeding season when conception occurred (Table 1). Failure to detect an effect of AMH concentration at weaning on subsequent fertility in beef heifers is in contrast with a study conducted with sheep. Lahoz et al. (2012) measured plasma AMH in 76 ewes at 3.6 months of age. The ewes were mated at 10 months of age, with those failing to conceive being mated again 4 months later. Results of that study indicated that fertility of ewes at first mating positively correlated with circulating AMH concentration at 3.6 months of age. The study concluded that a single AMH measurement performed on ewes at an early age was useful for selection of ewes with higher fertility potential at first mating.

Based on AMH concentration at breeding, heifers in the lowest quartile (Q1) had a lower ($P = 0.02$) AI pregnancy rate than heifers in other quartiles and conceived at a later cycle ($P = 0.03$) in the breeding season (Table 2). Studies have shown that heifers conceiving early in their first breeding season will continue to conceive early in subsequent breeding seasons, wean heavier calves, and be more productive throughout their life (Bellows and Staigmiller, 1994). Recently, Jimenez-Krassel et al. (2015) measured AMH on 11 to 15 month old Holstein heifers before first breeding, and then followed their reproductive performance and productivity through two lactations. Compared to heifers in higher AMH quartiles, heifers in the lowest AMH quartile on average had a productive herd life that was 196 days shorter, the lowest level of first lactation milk production, the lowest percentage for cows pregnant across all lactations, and the highest culling rate for poor reproduction.

Plasma AMH concentration in bovine females has been reported to remain relatively stable throughout the first year of life (Rota et al., 2002). In the current study, mean AMH in serum increased from 0.30 at weaning to

Table 1. Effect of serum anti-Mullerian hormone (AMH) concentration at weaning on cyclicity and pregnancy rate in beef heifers.

	Anti-Mullerian hormone quartile				
Item	**1**	**2**	**3**	**4**	***P*-value**
AMH range (ng/ml)	0.04 - 0.15	0.17- 0.24	0.25 - 0.38	0.40 - 0.99	
Cyclic at breeding (%)	8/17 (47.1)	11/18 (61.1)	8/16 (50.0)	9/17 (52.9)	0.855
Synchronized estrus (%)	10/17 (58.8)	14/18 (77.8)	10/18 (55.6)	13/17 (76.5)	0.354
AI pregnancy rate (%)	4/10 (40.0)	8/14 (57.1)	5/10 (50.0)	5/13 (38.5)	0.754
Overall pregnancy rate (%)	15/17 (88.2)	16/18 (88.9)	17/18 (94.4)	13/17 (76.5)	0.449
Mean conception cycle	1.33	0.81	1.24	1.00	0.523

AI = artificial insemination.

Table 2. Effect of serum anti-Mullerian hormone (AMH) concentration at breeding on cyclicity and pregnancy rate in beef heifers.

	Anti-Mullerian hormone quartile				
Item	**1**	**2**	**3**	**4**	***P*-value**
AMH range (ng/ml)	0.04 - 0.23	0.27 - 0.45	0.50 - 0.77	0.80 - 1.73	
Cyclic at breeding (%)	7/17 (41.2)	11/18 (61.1)	12/17 (70.6)	7/17 (41.2)	0.206
Synchronized estrus (%)	10/17 (58.8)	13/18 (72.2)	14/18 (77.8)	11/18 (61.1)	0.572
AI preg. rate (%)	1/10 (10.0)^{a}	7/13 (53.9)^{b}	6/14 (42.9)^{b}	8/11 (72.7)^{b}	0.021
Overall preg. rate (%)	14/17 (82.4)	16/18 (88.9)	16/18 (88.9)	16/18 (88.9)	0.919
Mean conception cycle	1.79^{a}	1.0^{b}	0.75^{b}	0.94^{b}	0.034

^{a,b} Within rows, numbers with different superscripts are significantly different ($P < 0.05$).
AI = artificial insemination.

0.56 ng/mL at breeding. It was also noted that the serum AMH concentration of some individual heifers either did not increase or actually decreased during this time. Therefore, heifers were assigned to quartiles based on the difference between AMH concentration at breeding and weaning (Table 3). None of the heifers became pregnant after AI in the lowest quartile (Q1), compared with 80% in the highest quartile (Q4; $P < 0.001$). Heifers in the lowest quartile also conceived at a later cycle in the breeding season than heifers in the other quartiles ($P = 0.01$).

In the study previously mentioned that was conducted with dairy heifers (Jimenez-Krassel et al., 2015) it was hypothesized that AMH concentration had a positive correlation with high antral follicle counts, fertility, and ovary function. The study confirmed that a single blood sample for AMH from breeding age dairy heifers could be used to select replacements and predict long-term reproductive performance of dairy heifers. Often reproduction is negatively correlated with other desirable traits. However, the results of Jimenez-Krassel et al. (2015) showed that AMH could be used to identify more fertile heifers without compromising milk production.

A study utilizing 1237 multiparous dairy cows of three different breeds determined if circulating AMH had a direct relationship with fertility during a planned 100-day breeding season (Ribeiro et al., 2014). The cows were synchronized, and either placed in timed insemination protocol or inseminated at estrus. Serum samples were collected on day eight of the estrous cycle for measurement of both AMH and progesterone. Concentrations of AMH were found to vary among the breeds of cows and those at different stages of lactation. Although no relationship was found between AMH levels for dairy cows enrolled in timed insemination, a positive correlation was found between AMH and pregnancy rates with dairy cows bred after detected estrus. In addition, Ribeiro et al. (2014) found pregnancy loss to be greater in cattle with lower AMH.

A study in goats reported that AMH could be used as a predictor of in vivo embryo production (Monniaux et al., 2011). Plasma AMH was measured in goats before follicle-stimulating hormone (FSH) treatments were given to stimulate follicular growth at the beginning of the breeding season, at the end of the breeding season, and during the anestrus period. High AMH was positively correlated with higher numbers of corpora lutea and embryo recovery. The study concluded that AMH could help predict the ability of goats to respond to the superovulatory treatment, as well as whether they will produce high numbers of transferable embryos. It was noted that the goats' plasma AMH concentrations gradually decreased after each embryo collection.

Results of the current study and others concur that AMH can be used as a predictor of fertility in replacement animals. This study concluded that AMH concentration at breeding and/or the change in AMH from weaning to breeding showed a positive correlation between AMH and fertility. Lahoz et al. (2012) measured AMH in sheep during the prepubertal period and found a correlation between AMH and fertility later in life. In goats, a positive correlation was measured between AMH and in vivo embryo production after superovulation. In both dairy heifers and mature cows, circulating AMH was shown to be positively correlated with fertility (Jimenez-Krassel et al., 2015; Ribeiro et al., 2014).

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Table 3. Effect of change in serum anti-Mullerian hormone (AMH) concentration from weaning to breeding on cyclicity and pregnancy rate in beef heifers.

	Anti-Mullerian hormone quartile				
Item	**1**	**2**	**3**	**4**	***P*-value**
AMH range (ng/ml)	-0.48 - 0.04	0.05 - 0.16	0.17 - 0.44	0.48 - 1.32	
Cyclic at breeding (%)	7/17 (41.2)	12/18 (66.7)	10/17 (58.8)	7/16 (43.8)	0.374
Synchronized estrus (%)	10/17 (58.8)	14/18 (77.8)	13/18 (72.2)	10/17 (58.8)	0.525
AI preg. rate (%)	0/10 (0.0)[a]	7/14 (50.0)[b]	7/13 (53.9)[b]	8/10 (80.0)[b]	>0.001
Overall preg. rate (%)	13/17 (76.5)	16/18 (88.9)	17/18 (94.4)	15/17 (88.2)	0.464
Mean conception cycle	1.92[a]	0.93[b]	0.88[b]	0.80[b]	0.014

[a,b] Within rows, numbers with different superscripts are significantly different ($P < 0.05$).
AI = artificial insemination.

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Efficiency of the nuclease I-*Sce*I in excising selectable marker genes from the plant genome

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Abstract

Gene stacking is a method used in biotechnology by which multiple genes can be placed at a single genomic site, thereby simplifying plant breeding. In this approach, DNA nucleases are used for excising selectable marker genes (SMG), which are the unneeded components of transgenic plants. The goal of this project is to evaluate the effectiveness of the nuclease I-*Sce*I in excising DNA in plants. Specifically, this study tests heat-inducible I-*Sce*I through the use of a heat-shock promoter (HS) in order to control SMG excision by heat application. The DNA plasmid containing a visual marker gene flanked by I-*Sce*I target sites and the heat-inducible I-*Sce*I gene has been created and confirmed. *Arabidopsis thaliana* plants have been transformed with the plasmid, which will be used for testing the efficiency of HS:I-*Sce*I in excising DNA from plant genomes.

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Meet the Student-Author

Eliott Pruett

I grew up in Summers, Arkansas and graduated as salutatorian from Haas Hall Academy in Fayetteville, Arkansas in 2012. I graduated from the University of Arkansas in May 2016 with a B.S. in Crop Science with minors in Agribusiness and Crop Biotechnology. This project was done to fulfill the research requirement of the Crop Biotechnology minor. In the spring of 2016 I presented this research at the University of Arkansas Gamma Sigma Delta and the Southern Section of the American Society of Plant Biologists undergraduate poster competitions and won first place in both. In 2014 I was an intern at Kansas State University in the Sustainable Bioenergy Research Experience for Undergraduates program; in 2015 I participated in the Adair Plant Pathology Internship at the University of Arkansas; and I was the 2016 Department of Crop, Soil and Environmental Science Outstanding Senior. I will be pursuing a graduate degree at the University of Arkansas starting in the fall of 2016, continuing my research with my faculty mentor, Vibha Srivastava. I would like to thank Vibha Srivastava and Soumen Nandy for this opportunity and for their instruction.

Introduction

Selectable marker genes (SMG) are an invaluable tool for generating transgenic plants; however, their presence in the transgenic crops is highly undesirable, and complicates the biotechnology risk assessment and regulatory procedure. Furthermore, due to the limited number of SMGs available to carry out plant transformation, they have to be reused when attempting to stack traits. This requires the use of DNA recombinases or nucleases to remove the selectable marker gene once it is no longer needed. Recombinases have been used both to add traits and remove SMGs; however, this can only be done once with each recombinase due to the reversibility of the process (Dale and Ow, 1991; Kumar and Fladung, 2001; Nandy et al., 2015). The alternative to using a recombinase is to use nucleases, which carry out irreversible forward reactions.

This alternative method is studied here using I-*Sce*I: a nuclease that causes double-stranded DNA breaks at a specific 18 base pair site (5'-TAGGGATAA^CAGGGTAAT-3'). When double-stranded breaks occur on both sides of a gene, the gene is deleted from the chromosome and the cell repairs the break. During this process, insertions and/or deletions (indels) can occur at the repair site (D'Halluin et al., 2013; Puchta and Fauser, 2014; Voytas 2013) making the site resistant to the nuclease and rendering the process irreversible. It is essential to know how frequently indels occur since large indels may alter the function of adjacent genes while small indels are acceptable. The promoter regulating I-*Sce*I is the soybean heat-shock protein 17.5E gene promoter (HS). It is inactive at 25 °C (room temperature) but highly active at 42 °C (Czarnecka et al., 1992). This heat-inducible I-*Sce*I will eliminate the need to retransform or cross plants for introducing I-*Sce*I activity, making the process much more efficient.

The overall goal of this project is to evaluate the efficiency of heat-inducible I-*Sce*I in excising a SMG from the genome of the model plant, *Arabidopsis thaliana*. The green fluorescence protein (GFP) gene was targeted for excision. The GFP expression is easily monitored as it emits green fluorescence under blue light. For testing I-*Sce*I efficiency in DNA excision in a plant genome, it is a prerequisite to develop transgenic plants containing a gene (GFP) flanked by I-*Sce*I sites, and the heat-inducible I-*Sce*I gene (HS:I-*Sce*I). These genes must be inserted into a DNA plasmid, which can then be inserted into the plant genome. This study developed these essential genetic resources, which will be used for evaluating I-*Sce*I efficiency in excising DNA from *Arabidopsis* genome.

The specific objectives of this project are to 1) build and confirm a DNA construct containing HS:I-*Sce*I, and 35S:GFP flanked by I-*Sce*I sites, in binary vector backbone; and 2) introduce the construct into *Arabidopsis* by *Agrobacterium*-mediated transformation. Completing these objectives is this first step towards testing HS:I-*Sce*I efficiency.

Molecular Strategy

In order to test heat-inducible I-*Sce*I, it is necessary to have a plasmid containing HS:I-*Sce*I and I-*Sce*I sites flanking the portion of DNA that is to be excised. The portion of DNA chosen was 35S:GFP, because it allows visual confirmation that the gene is active when inserted into plants. Additionally, the neomycin phosphotransferase (NPT) gene was inserted in order to confer resistance to the antibiotic kanamycin, which allows selection of transformed plants containing the plasmid. The HS:I-*Sce*I gene, 35S:GFP gene, 35S:NPT gene, and an I-*Sce*I site were already available but they needed to be combined to develop a structure (pEP4b) shown in Fig. 1, and cloned into a *Agrobacterium* binary vector (i.e. plasmid) for plant transformation. The 35S and HS are constitutive and heat-shock promoters, respectively, and the transcription terminator used in each of the genes is that of the nopaline synthase gene. When the plants carrying this construct are heat-shocked, I-*Sce*I will be activated, which will create double-stranded breaks (DSB) at I-*Sce*I sites leading to the deletion of GFP gene. The broken ends will be repaired by the cell incorporating indels at the repaired site (see Fig. 2). The deletion of GFP gene can be monitored by the disappearance of green fluorescence. Additionally, a primer pair can be used to determine excision via polymerase chain reaction (PCR; see Figures 1 and 2).

Materials and Methods

DNA Construction and *Agrobacterium* Transformation

The first objective in this study was to create a DNA construct containing HS:I-*Sce*I and the GFP gene flanked by I-*Sce*I sites. The GFP gene is regulated by the 35S promoter from the cauliflower mosaic virus's 35S RNA gene. Cloning was done using standard molecular biology techniques using *Escherichia coli* cells. The *Agrobacterium* binary vector pPZP200 was used to construct the plasmid that contains a gene to confer resistance in bacteria to the antibiotic spectinomycin. Each gene was individually in-

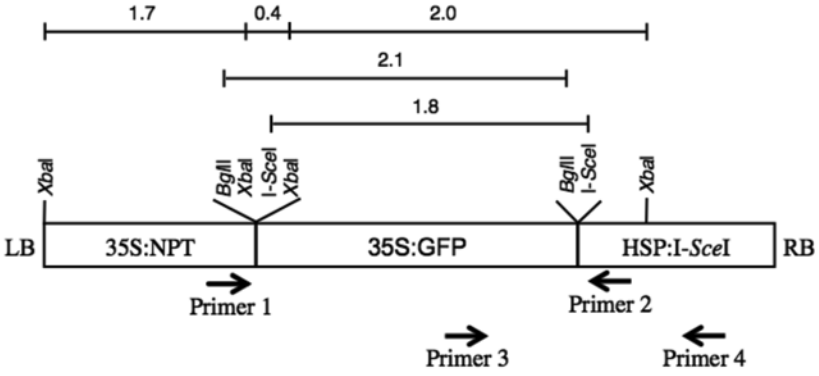

Fig. 1. pEP4b DNA construction in the binary vector pPZP200 (*Agrobacterium tumefaciens* vector) used to generate transgenic *Arabidopsis* lines. The vector contains 35S:NPT, 35S:GFP, and HSP:I-*Sce*I. LB and RB refer to the left and right borders of the *Agrobacterium* T-DNA. The sequence between these borders will be inserted into the *Arabidopsis* plants by the *Agrobacterium*. The location of polymerase chain reaction primers and restriction fragment sizes are shown.

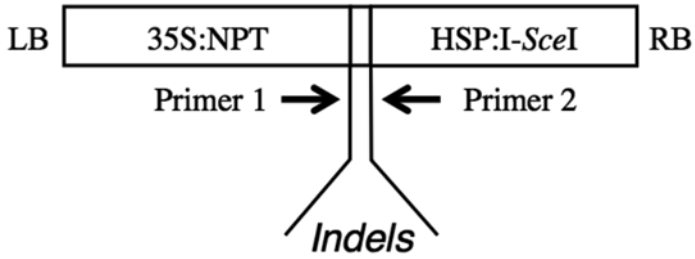

Fig. 2. Activation of heat-shock promoter leads to I-*Sce*I expression, which creates double-stranded breaks at the target I-*Sce*I sites. As a result, 35S:GFP gene is deleted and the broken ends are repaired by cellular DNA recombination process generating insertion-deletion (indels) at the cut site.

serted into the plasmid. This was done by digesting the plasmid and the gene by the same restriction enzymes, combining them in a single tube, and joining them together by DNA ligase. Following ligation, the new plasmid was inserted into a solution containing *E. coli* cells. This solution was then placed in a hot water bath, during which the *E. coli* cells would take up the new plasmid. The transformed *E. coli* was grown on media containing spectinomycin so that only the cells containing the plasmid would be able to grow. The plasmid was multiplied by the *E. coli* and then isolated. This process was repeated with each gene until they were all inserted into the pPZP200 vector. The final plasmid, pEP4b, was verified by restriction digestions, PCR and DNA sequencing.

Plant Transformation

The final plasmid, pEP4b, was introduced into *Agrobacterium tumefaciens* (strain GV3101) by electroporation using the Bio-Rad Gene Pulser, which was then used to transform *Arabidopsis thaliana* (Col-0 ecotype) plants. Plant transformation was done using the floral-dip method described by Clough and Bent (1998) to obtain transgenic seeds (T1). Seeds were collected from the dipped plants and grown on media containing the antibiotic kanamycin so that only plants containing pEP4b were able to grow.

The plasmid pEP4b was also inserted into rice callus to verify that GFP expression would occur in plant cells. Callus from the cultivar Nipponbare was grown on media, and pEP4b DNA was introduced by biolistic delivery method. The callus was incubated in a growth chamber for 48 hours before observing with a stereo-microscope for GFP fluorescence under blue light.

Results and Discussion

The plasmid, pEP4b, has been developed for testing the efficiency of I-*Sce*I in excising DNA in plant genome. It contains a DNA construct consisting of 35S:GFP flanked by I-*Sce*I sites, a selectable marker gene, 35S:NPT, for isolating transformed *Arabidopsis* lines, and the heat-inducible I-*Sce*I. The construct and the order of the genes is shown in Fig. 1. This construct was cloned into the binary vector, pPZP200 backbone, to generate pEP4b of approximately 12,000 base-pairs (12 Kb) size. The structure of pEP4b was verified by restriction digestion, which is a standard molecular biology technique that uses restriction enzymes to cut DNA at specific sites. The enzymes used were *Bgl*II, *Xba*I and I-*Sce*I. The sites where they cut the DNA construct are shown in Fig. 1. Digesting with *Bgl*II should result in two DNA fragments, with lengths of 2.1 Kb (construct) and 9.9 Kb (backbone). Digesting with I-*Sce*I should result in two DNA fragments as well, with

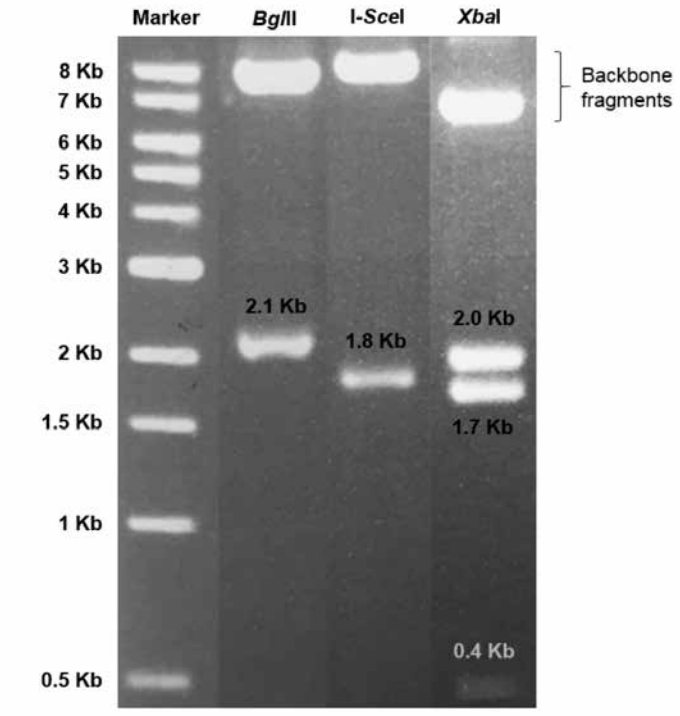

Fig. 3. Confirmation of pEP4b by restriction-digestion and gel electrophoresis. DNA marker lanes indicate DNA size standards, restriction enzymes are indicated on each lane, and the backbone fragments or the fragment sizes expected from the pEP4b construct (see Fig. 1) are given.

lengths of 1.8 Kb (construct) and 10.3 Kb (backbone). Digesting with *Xba*I should result in four DNA fragments, with lengths of 0.4 Kb (construct), 1.7 Kb (construct), 2.0 Kb (construct) and 7.8 Kb (backbone). As shown in Fig. 3, all of these fragments were obtained from the digestion of pEP4b with these restriction enzymes.

Note that digesting with I-*Sce*I is effectively what should happen when HS:I-*Sce*I is activated in plant cells. This confirms that the I-*Sce*I sites are functional. In order to test the functionality of the 35S:GFP gene, rice callus was bombarded with pEP4b using a gene gun and observed for GFP expression. The callus showed GFP expression, indicated as bright green spots, confirming the activity of GFP in plant cells (see Fig. 4a). This completes the first objective.

The plasmid was subsequently introduced into the *Agrobacterium tumefaciens* strain GV3101, which was used to transform *A. thaliana* by floral-dip method. The seeds (T1) from dipped plants were collected, and plated on seed germination media supplemented with kanamycin (50 mg/L). Seventeen T1 seedlings were able to grow on media and were confirmed by PCR to contain pEP4b. Figure 4b shows PCR amplification of pEP4b region spanning Primer 3 and Primer 4 (see Fig. 1) in the selected T1 plants. All 17 T1 plants have been transferred to pots containing a growing medium for seed production. This completes both objectives of this study. These plants will be further characterized by molecular techniques for the structure of pEP4b inserts and heat-induced activity of I-*Sce*I in each T1 line to select 2 -3 lines for further studies.

Acknowledgements

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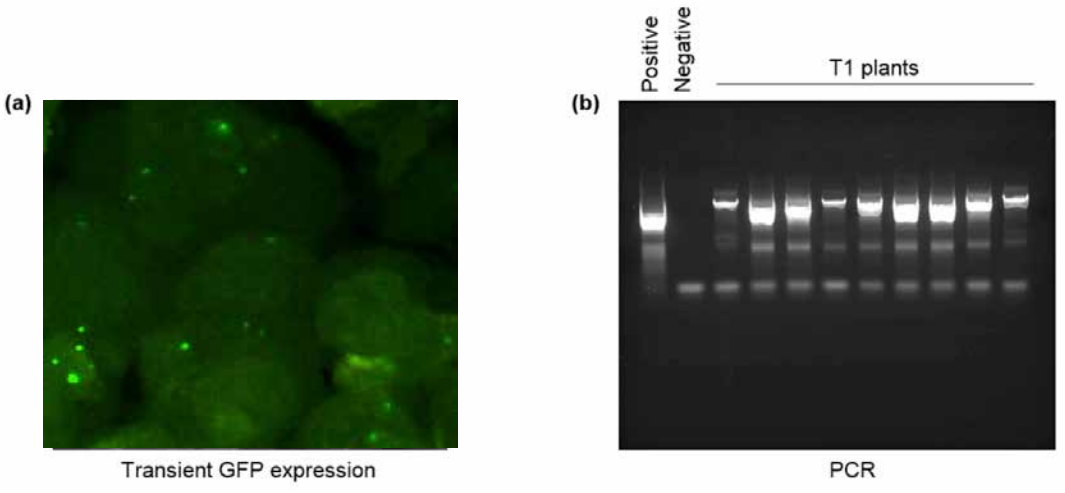

Fig. 4. Transient GFP expression in rice callus bombarded with pEP4b. The cells expressing GFP are seen as bright green spots. (b) Polymerase chain reaction (PCR) confirmation of *Arabidopsis* T1 plants transformed with pEP4b. Positive and negative controls refer to pEP4b and water controls, the presence of ~1 kb amplicons in 9 representative T1 lines is shown.

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Current status of the Northern Saw-whet Owl (*Aegolius acadicus*) in Arkansas

Mitchell L. Pruitt* and Kimberly G. Smith†

Abstract

The secretive Northern Saw-whet Owl (*Aegolius acadicus*) is believed to be much more widespread during fall and winter than previously thought. Of the few places in the southern United States conducting research on this species, all have been successful at capturing birds. A total of 12 historic records existed for Arkansas until our work began in fall of 2014. The first confirmed record was in 1959 and the most recent, prior to this research, was in 2010. Over the course of two field seasons, we captured and banded 24 Northern Saw-whet Owls in rural Madison County. All birds were mist-netted along a trail, in woodland composed of pine and cedar with fairly dense undergrowth. Two were captured during our 2014 season after a late start and 22 were captured in 2015, likely the result of an earlier start. Comparing our data to that of several other banding operations in the south, it would appear that the peak of migration in Arkansas is late October through early November, with capture rates dropping by early December. Of the birds captured, all but one was female, the most common sex this far south. A variety of age classes were identified, with a fairly even distribution of hatch-year, second-year, and after-second-year birds. Exactly from where the saw-whets are migrating is unknown, although several foreign recoveries in Missouri and four recoveries in Arkansas suggest they are coming from the western Great Lakes region. Once considered a vagrant, based on this research, the saw-whet appears to be a fall migrant to the state of Arkansas.

* Mitchell L. Pruitt is a May 2016 honors program graduate with a major in Environmental, Soil, and Water Science.
† Kimberly G. Smith, the faculty mentor, is a distinguished professor in the Department of Biological Sciences.

Meet the Student-Author

Mitchell Pruitt

I was born and raised in Jonesboro, Arkansas and graduated from Valley View High School in 2012. I graduated with honors from the University of Arkansas in May 2016 with a B.S. in Environmental, Soil, and Water Science. I developed a love of all things outdoors, especially birds, at a young age. During the summer, I teach at an ecology camp for 11-12 year olds, the same camp that inspired who I am today. When I am not doing schoolwork, I can be found birding in Arkansas and beyond. I am also a nature photographer, interested in wildlife (especially birds), herps (reptiles and amphibians), and more. My hobbies have snowballed into lifelong passions and, hopefully, a career. After graduating, I will complete a Master of Science degree in biology, working specifically with birds of prey. For two years, I have actively researched the Northern Saw-whet Owl, under the direction of Kimberly Smith in the Department of Biological Sciences. We documented the species' occurrence in Arkansas, where it was not previously known to regularly exist. It has been an amazing road and a great learning experience! I would like to thank my thesis advisor, Smith, and my thesis committee: Lisa Wood and Thad Scott. Everyone else who helped in making this research run smoothly has been a great resource, including Dana Ripper, Missouri River Bird Observatory, Matthew Miller and the staff of the Ozark Natural Science Center, and all banding volunteers, especially Melyssa St. Michael, Jacqueline Guzy, Joe Neal, David Oakley, and Meredith Swartwout.

Introduction

In eastern North America, Northern Saw-whet Owls (*Aegolius acadicus*) are primarily a denizen of the boreal forests of Canada during the breeding season, but birds migrate south in fall into the United States (Confer et al., 2014), sometimes in large "invasions" (Brinker et al., 1997). However, its distribution is poorly known in the southern part of the United States. Recently, attempts to capture birds during fall migration have been successful in Missouri (D. Ripper, unpubl. data) and Alabama (R. Sargent, unpubl. data), as was an earlier attempt in South Carolina, primarily in 1999 (W. Hilton, pers. comm.).

Between 1959 and 2010, there were 12 reports of saw-whets in Arkansas, most of which occurred in November and December (Arkansas Audubon Society, James and Neal, 1986) (Fig. 1). These records were scattered, but were mostly north of the Arkansas River, with an emphasis on the Ozarks, Crowley's Ridge, and the tip of the Ouachita Mountains at Little Rock (Fig. 2).

A saw-whet was photographed by Arkansas Gazette photographer, Larry Obsitnik, on a no parking sign during the day in Little Rock on 7 November 1969. It appeared on the front page on 8 November (Fig. 3). A detailed description of the history of saw-whets in Arkansas is presented in Pruitt and Smith (2016).

Based on the success of capturing birds in Missouri and Alabama, the objective of this study was to attempt to document the occurrence of saw-whets in Arkansas during fall and winter, using mist-nets and audio lures for the first time. Prior to our research, saw-whets were considered a rare bird within the state of Arkansas (James and Neal, 1986). James and Neal (1986) concluded that due to their nocturnal habits and secretive nature, saw-whets might be more common in Arkansas than records suggested. Nonetheless, our expectation was that we would capture no saw-whets.

Materials and Methods

This research used standard methods produced by a group of researchers in the northeastern United States (Project Owlnet, 2016). Before beginning, banding permits were acquired from both the national and state governments, as well as from the particular organization on whose property we were netting. Standard equipment included four 12-meter mist nets with 60-mm mesh, an audio lure to draw birds into the net area, and tools for processing upon capture. A typical night consisted of being in the field from 7:00 PM until 12:00 AM or later.

During fall and winter months, saw-whets seem to have a preference for woodland with a thick understory, ideally cedar or other coniferous component. Our field station

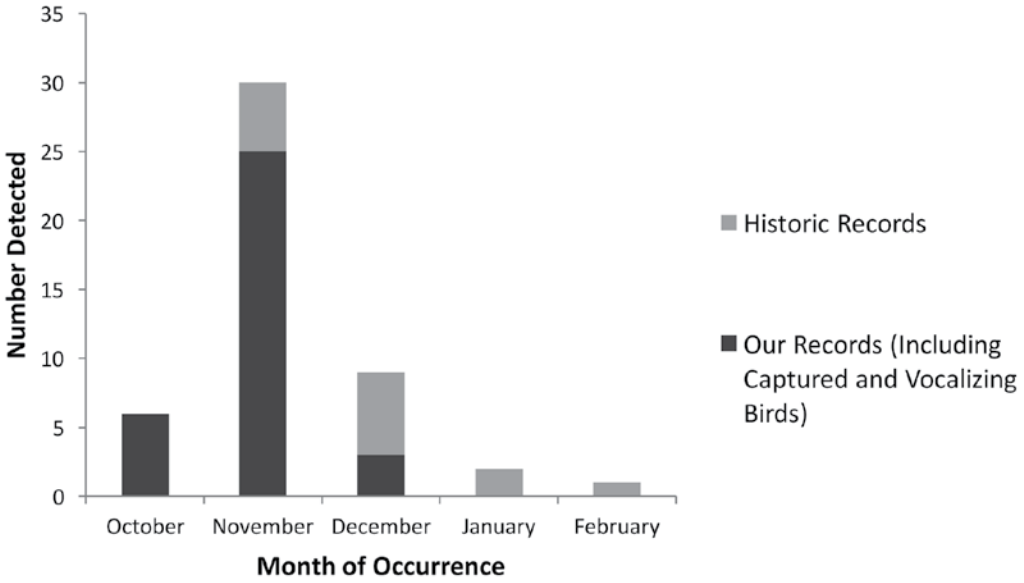

Fig. 1. Arkansas Northern Saw-whet Owl records from 1959 through 2015 by month. The historic records are from the Arkansas Audubon Society database and include the two game-camera records from Madison County in December 2014 and January 2015 mentioned in the text.

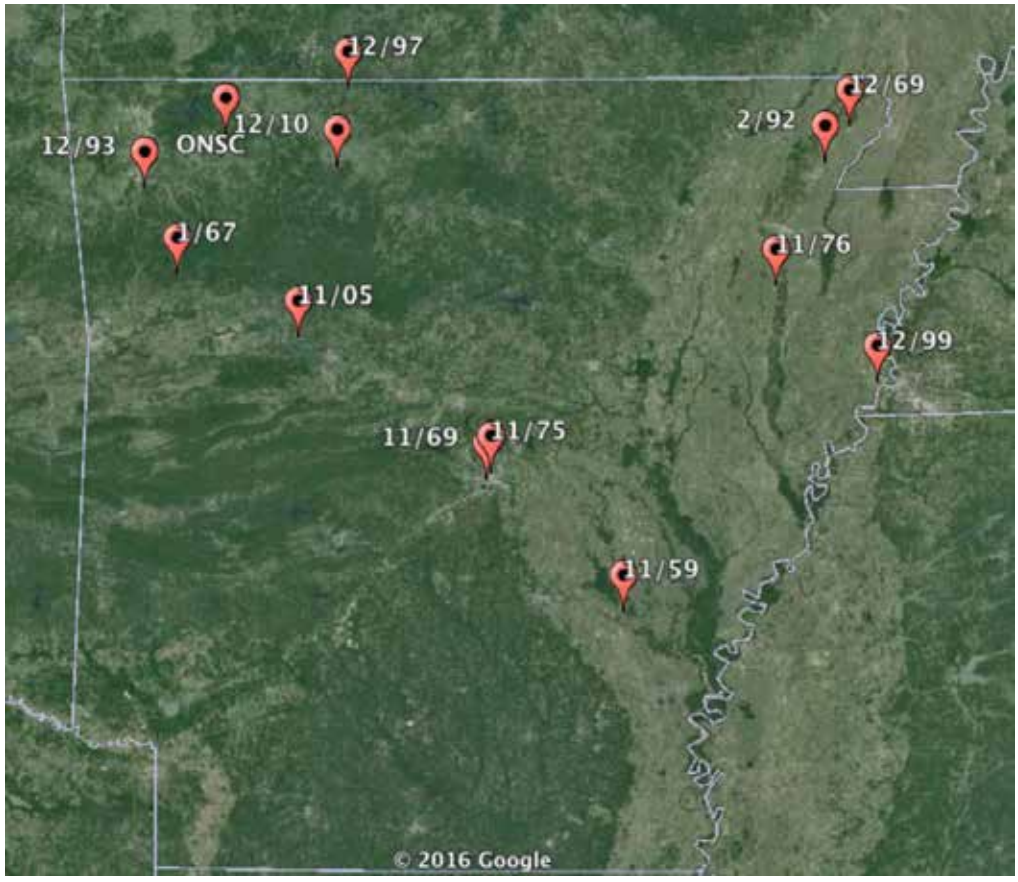

Fig. 2. Distribution of the first 12 records from Arkansas of Northern Saw-whet Owls with the month and year of each sighting. ONSC refers to the location of this field study, Ozark Natural Science Center. Note that most sightings are associated with heavily forested areas on the background map.

was located at the Ozark Natural Science Center (ONSC) in rural Madison County, Arkansas, where the habitat is a mixture of pine/deciduous upland with a thick cedar understory. Four mist nets were arranged in a line down a trail through the cedars. The audio lure was placed at the center of this arrangement and played continuously during time afield. The use of an audio lure began in 1986, at the Little Suamico Ornithological Station near Green Bay, Wisconsin, as a method to increase saw-whet captures (Erdman and Brinker, 1997). The lure was played on a FoxPro® brand predator caller programmed with several call types of the saw-whet. Call types played included the breeding male's toot, toot, toot as well as a whine call, which is often given during migration (Weidensaul, 2015). Calls were obtained from the Stokes Field Guide to Bird Songs CDs, by Donald and Lillian Stokes, and are part of the standard procedures for capturing saw-whets.

Upon capture, a bird was taken inside a building for processing. Processing involved sexing, ageing, and banding. Like many raptors, saw-whet owls exhibit reverse sexual dimorphism meaning females are, on average, slightly larger than males (Weidensaul, 2015). Accurate sexing of saw-whets can only be done by comparing a bird's closed wing-chord (CWC; maintains the wing's natural arc) and its weight. Brinker (2000) created a chart for sexing with ease; it has a >95% probability for accuracy. On average, females have a CWC of 120-141 mm and weigh 88 to greater than 93 g, while males have a CWC of 120-135 mm and weigh less than 78-88 g. All birds were weighed in a mesh banding bag using a Pesola spring scale.

Ageing saw-whets involves the use of ultraviolet (UV) light to fluoresce porphyrin pigment on the ventral surface of flight feathers (Primaries: P1-P10; Secondaries: S1-S12). In saw-whets, this pigment is pink when fluoresced by UV light. Once exposed to sunlight, porphyrins begin to fade making different ages of feathers fairly distinct. New feathers fluoresce bright pink, middle-aged feathers are light pink, and old feathers may not show any pink (Weidensaul et al.,

Fig. 3. Photograph of Northern Saw-whet Owl by Larry Obsitnik taken the day before it appeared on the front page of the Arkansas Gazette on 8 November 1969. He had no idea what the owl was and was making a joke about the owl not being able to read the sign. Doug James identified the bird from the picture in the newspaper and obtained a copy of the picture for the Arkansas Audubon Society files. (Photo courtesy of Lyndal York).

2011). Three distinct age classes can be identified using this method (Fig. 4). Hatch year (HY) birds exhibit flight feathers of a single age. Second year (SY) individuals exhibit two distinct ages of flight feathers. After second year (ASY) birds exhibit three or more distinct ages of feathers (Pyle, 1997). After a saw-whet's second year, its age cannot be identified more specifically unless it was previously banded. Finally, captured birds were banded using a size four short federal band, and released into the night.

Capture rates were calculated for the fall 2015 banding season based on birds captured per 100 net-hours, the standard way of reporting banding effort for saw-whets. Typically, 4 nets were open for 4 hours each night, or 16 net-hours per night. The season capture rate was calculated from the night with the first capture to the night of the last capture.

Results and Discussion

Over the course of two field seasons, a total of 24 saw-whets were captured and banded at the Madison County field site. Ten more were detected vocally, resulting in 34 recorded individuals. In 2014, we did not begin netting efforts until 20 November due to issues in the permitting process. Even so, two individuals were captured and two others detected vocally. The first saw-whet owl was captured on 21 November and was in the company of another individual that was not captured. One of these birds responded to the audio lure earlier the same night. A second bird responded to the audio lure on 6 December, but was not captured. However, an individual was captured the following night of 7 December. Efforts continued through January 2015 and sporadically into February with no captures or vocal detections. With insight from researchers in central Missouri, the second field season began earlier, on 25 October 2015, and continued through 3 December 2015 (D. Ripper, pers. comm.). During this time, 22 saw-whets were captured and banded; eight others were detected vocally (Fig. 5). The 2015 field season consisted of 23 total nights afield, or 257.3 total net-hours. Of these 23 nights, 10 nights had captures (43.5%) and 12 nights had captures or vocal detections (52.2%). On 75.0% of nights when saw-whets were captured or detected, there were more than two captures or detections per night.

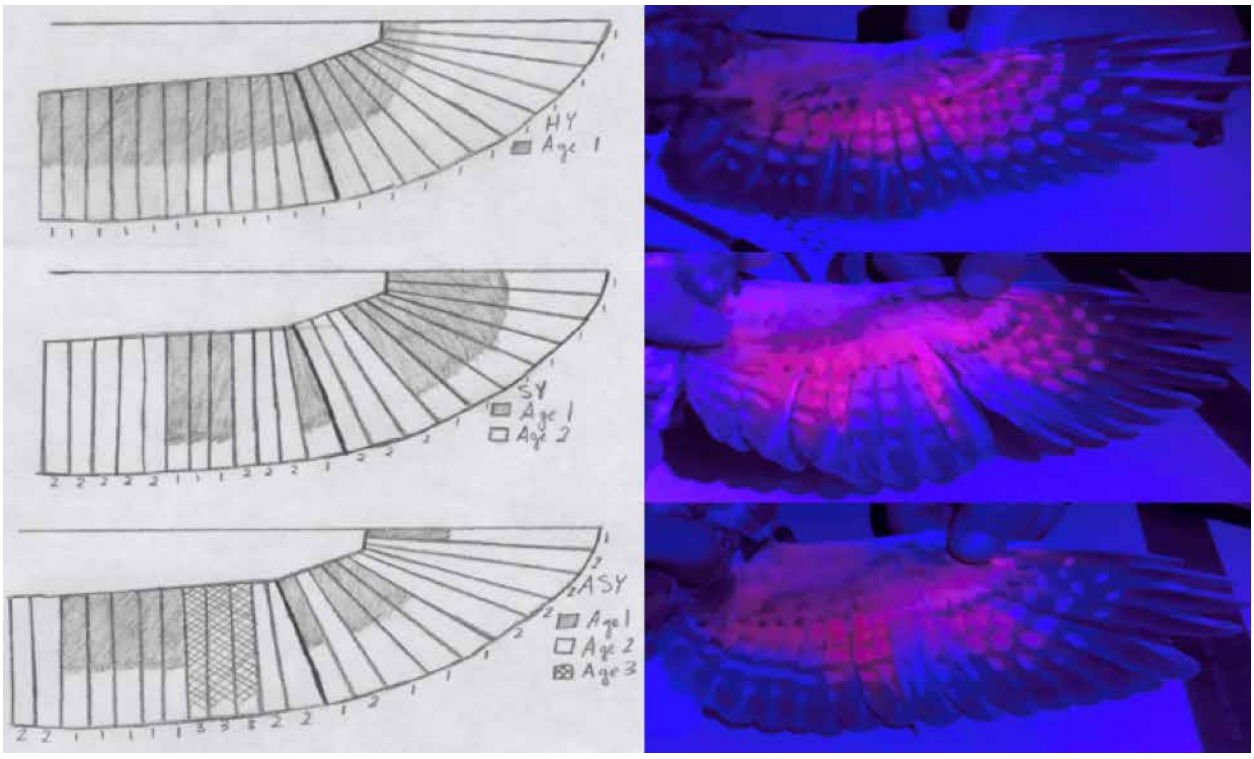

Fig. 4. Age classes of the saw-whet owl based on fluorescence of porphyrin on the underwing. Top: Hatching-year (HY) bird with uniform color indicating that all feathers are new. Middle: Second-year (SY) bird with 2 different kinds of feathers: new feathers are bright while second-year feathers are faded. Bottom: After second-year (ASY) bird with 3 different kinds of feathers: new feathers are bright, second year feathers are paler, and third year feathers hardly fluoresce. (Photos and drawings by Mitchell Pruitt 2015).

The sex ratio of the birds was skewed towards females. Only one individual out of 24 total captures was identified as a male; 23 were females. The single male was captured 21 November 2015 and was aged as a hatch-year bird. The male had a closed wing chord (CWC) of 136 mm and weighed 80 g. The average CWC of captured females was 141.9 mm (+/- 0.57 SE) with a range of 138-146 mm. The average weight of captured females was 90.9 g (+/- 1.16 SE) with a range of 80-105 g.

The age distribution was evenly distributed among the three identifiable classes: HY (n = 8), SY (n = 7), ASY (n = 8), and fourth year (n = 1). A saw-whet captured on 7 November 2015, at ONSC, was previously banded at the Linwood Springs Research Station near Stevens Point, Wisconsin on 17 October 2013. It was banded as a second year bird, meaning it was in its fourth year at the time of recapture at our field site. Comparing the ages of captured saw-whets to date of capture, it would appear that hatch-year birds arrive at about the same time as adults (Fig. 6).

The capture rate for 2015 was 8.6 birds per 100 net-hours. Records from this research were compared to Arkansas' historic records and show a peak in migration during November; more specifically the first two weeks in November (Figs. 1 and 5). Interestingly, most of the captures seemed to occur during the hours of 9:00 PM-10:00 PM and again around midnight (Fig. 7).

During the 2015 season, two captures were foreign recaptures (FRs), meaning they were banded somewhere other than the ONSC field site. First was the aforementioned 4-year-old bird banded (0914-53397) in October 2013 in Stevens Point, Wisconsin and captured at ONSC in November 2015 (Fig. 8). This owl was an underweight (80 g) female with a CWC of 144 mm, aged fourth year. The second FR occurred on 21 November 2015 and was banded (0914-99385) on 30 September 2015 at Hawk Ridge Bird Observatory near Duluth, Minnesota (Fig. 8). The distance between the two research sites is 1186 km indicating the bird averaged at least 23 km/night. This ASY female weighed 91 g and had a CWC of 145 mm. There was also a local recapture during our 2015 season. This saw-whet, a HY female, was banded at the ONSC field site on 7 November 2015. She weighed 86 g, slightly underweight. The bird was recaptured the following night, 8 November, weighing 91 g and had dried blood on her beak and talons suggesting that she had eaten.

From this research, we are able to conclude that the Northern Saw-whet Owl is, in fact, more common in Arkansas than previously thought, at least during fall migration. With only 12 confirmed records throughout the history of bird documentation in Arkansas, capturing the species was not expected. However, with 24 total captures, and 10 additional vocal detections, it is reasonable to think the species previously went undetected, probably due to their secretive nature.

All but one of our captured saw-whets were females. Males are captured with much less frequency further south of the species' normal range (Brittain et al., 2009, Beckett

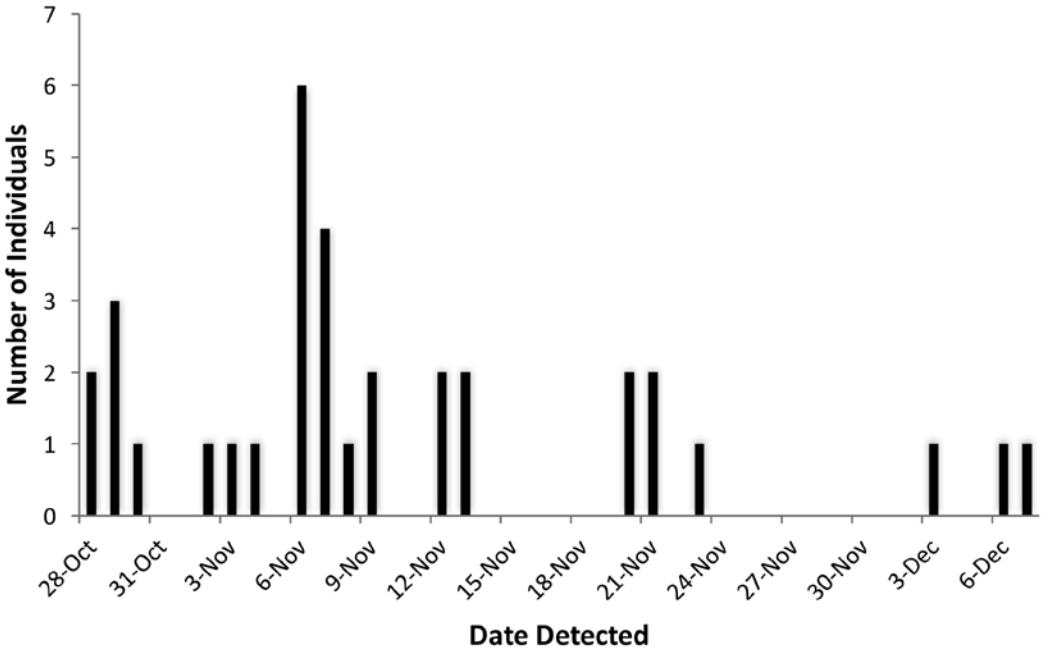

Fig. 5. Saw-whet detections (capture or vocal) at Ozark Natural Science Center during November and December 2014 and October to December 2015 (combined).

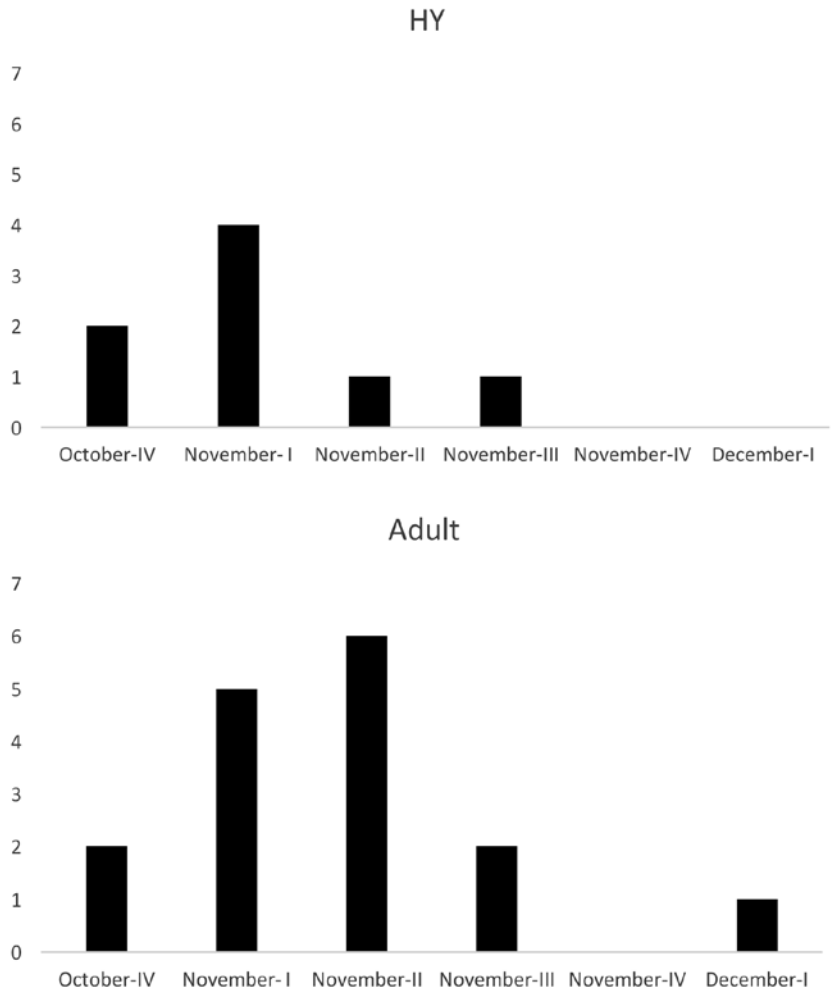

Fig. 6. Arrival of hatch-year (HY) and adult Northern Saw-whet Owls by week from the 4th week of October through the first week of December. Hatch-year birds appear to arrive at the same time as adults.

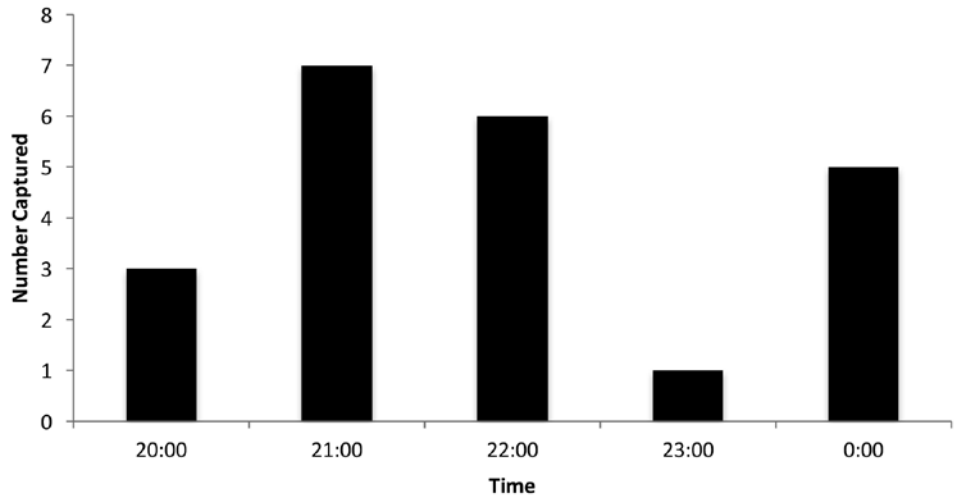

Fig. 7. Saw-whets captured per hour at Ozark Natural Science Center during November and December 2014 and October to December 2015.

and Proudfoot, 2012). Brinker et al. (1997) suggested this is because males do not stray as far from prime breeding habitat, allowing for quicker reoccupation in spring when they are vying for precious cavities for nesting. Or the larger and heavier females may have dietary requirements that are met further south (Weir et al., 1980, Beckett and Proudfoot, 2012). Such differential migration is not uncommon in birds and has been documented in the Boreal Owl (*Aegolius funereus*), a close relative of the saw-whet (Brinker et al., 1997).

Based on only one full field season, we cannot attribute much to the equal distribution of age classes that were found. Brittain et al. (2009) found that the number of HY birds fluctuated annually from about 30% to 50% in southern Indiana. At northern locations, HY birds usually appear first in the fall, but the limited data suggest that they arrive at the same time as adults in northwestern Arkansas.

Capture rates in our 2015 season started in late October, peaked during the first few weeks of November, and decreased to no captures after the first week of December. This trend is also similar to that of Missouri (D. Ripper, unpubl. data) and slightly before that of northern Alabama, where captures continued into January (R. Sargent, unpubl. data). This difference in Alabama might be because those birds are following a different migratory pathway (see below). This peak in early November coincides exactly with the prediction from the model presented in Beckett and Proudfoot (2011) for a northern latitude of about 36 degrees. Our

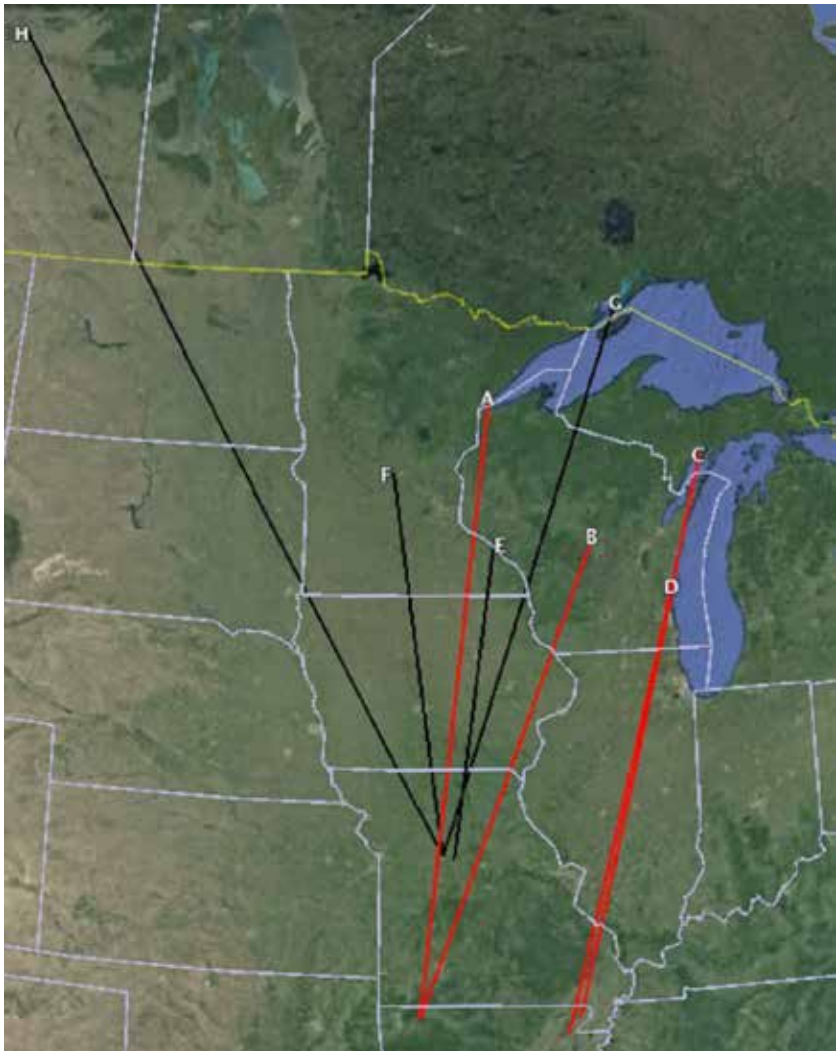

Fig. 8. Banding recoveries from Arkansas (red) and from the Missouri River Bird Observatory (black). Most birds appear to be coming from the western Great Lakes region. Key (banded, recovered): A (9/15, 11/15), B (10/13, 11/15), C (10/90, 2/92), D (11/69, 12/69), E (10/15, 11/15), F (10/15, 11/15), G (?/13, 10/14), H (9/12, 10/12).

results agree with those authors, that fall migration of saw-whets is a uniform front that moves southward as fall progresses.

Weather conditions also appear to play a role in successfully capturing saw-whets. The nights that most birds were captured followed cold fronts from the north, suggesting that migrating birds were riding those fronts. Brittain et al. (2009) also caught more birds in southern Indiana following the passage of fronts and on nights with calm winds (see also Weir et al., 1980). Nights with full moons are typically unproductive (Speicher et al., 2011), because birds can see the mist nets and/or are wary of larger, predatory owls, such as Barred Owls (*Strix varia*), which were commonly heard calling at the ONSC field site. However, four captures were made in late October when the moon was an 85% full waning gibbous. This was probably due to leaves still being on the trees, darkening the forest near the nets.

Based on four foreign recovery data from Arkansas and three of four from Missouri, it would appear that the saw-whets migrating to the region are coming from the western Great Lakes region (Fig. 8). Four recoveries from Arkansas include two birds banded in Wisconsin, one from Duluth, Minnesota, and another banded in the Upper Peninsula of Michigan. Three recoveries from Missouri include birds banded in Kellogg, Minnesota, Collegeville, Minnesota, and Silver Islet, Ontario (Fig. 8). (The other Missouri bird came from Prince Albert, Saskatchewan, far to the northwest.) These data appear to establish a heretofore unknown migration route for saw-whets, flying south or southwest from the western Great Lakes to the Ozarks (see Confer et al., 2014). Birds in Alabama could possibly be coming from somewhere other than the western Great Lakes, like down the Appalachian Mountains, which might explain the longer banding season there.

The fact that 10 vocalizations were observed during this research should also be noted, as vocalizations are thought to be uncommon outside the species' breeding season. The saw-whet is so-named by its vocalizations reminiscent of whetting a saw, although it is unknown specifically for which call it was named (Weidensaul, 2015). During the 2014 and 2015 field seasons, several different vocalizations were documented. One of the vocalizations played by the audio lure is the male's territorial toot, toot, toot call. No response was heard to this call because it is rarely heard outside breeding season. The second vocalization played by the audio lure is an eerie, drawn out whine call that is heard most frequently in fall and could be a contact call used during migration to locate other individuals (Rasmussen et al., 2008). Most often a response to the whine call was heard, but we documented several other vocalizations as well. Another common call heard during field research was a quick ksew or chirping note. This was often elicited by flushing birds while checking nets. Both the ksew and squeak seemed to be given by agitated individuals. Ksew notes were also heard while listening from a distance, meaning they were probably given off in agitation towards other individuals as well. On one occasion, a two note, squeaking alarm call was heard from a flushing bird. On another occasion, two individuals were heard high up in a tree giving a series of soft chirping notes, seemingly talking back and forth to each other. The saw-whet is still vastly understudied outside migration, making it difficult to understand the social context behind most of their vocalizations.

Based on the scattered historic records, it would appear saw-whets could be found throughout Arkansas. There are also other large tracts of suitable cedar habitat in northwestern Arkansas. Thanks to publicity of this project, we were contacted by Becky Christenson, who had 2 images of a saw-whet owl from a trail camera that she had set up on her property approximately 16 km south of Kingston (Madison Co.) on County Road 3655. The first image was taken at night on 23 December 2014 and the second image (presumably both images were of the same bird) was taken on 12 January 2015. Her property is about 32 km due south of the research site at Ozark Natural Science Center. This is likely just one of several unknown and unreported individuals.

After early December, our capture rates drop to zero and saw-whets seem to vanish. We continued banding operations into January and early February of 2015, but caught or heard no birds. The banding station in Missouri also typically shuts down after the first week of December as they do not catch any birds after that time (D. Ripper, pers. comm.). However, sporadic records in Arkansas from December to February suggest that some individuals may spend the winter here. Is this suggestive of the population as a whole or just these few individuals?

There are several possibilities: First, they could be going further south, but there are no records in southern Arkansas and almost none in Louisiana. Second, they could be spending winter in the Ozarks, but they no longer respond to audio lures after late November. Third, they could be returning north in December, but that seems to oppose the logic behind migration. Or they could be doing something completely different, like wandering throughout winter, as has been found in Snowy Owls (*Bubo scandiacus*) (Norman Smith, pers. comm.).

From this study, it can be concluded that Arkansas is most likely in a previously unknown migratory pathway for the saw-whet owl. This research has more than doubled the state's previous 12 records in just two field seasons. One thing is certain, a species with such gaps in its natural history is dangerous in today's ever-progressing world. The goal of this research, and future projects, is to learn more about the migration of the Northern Saw-whet Owl in Arkansas and the southern region. Further research will be imperative to this secretive species' conservation in the future.

Acknowledgements

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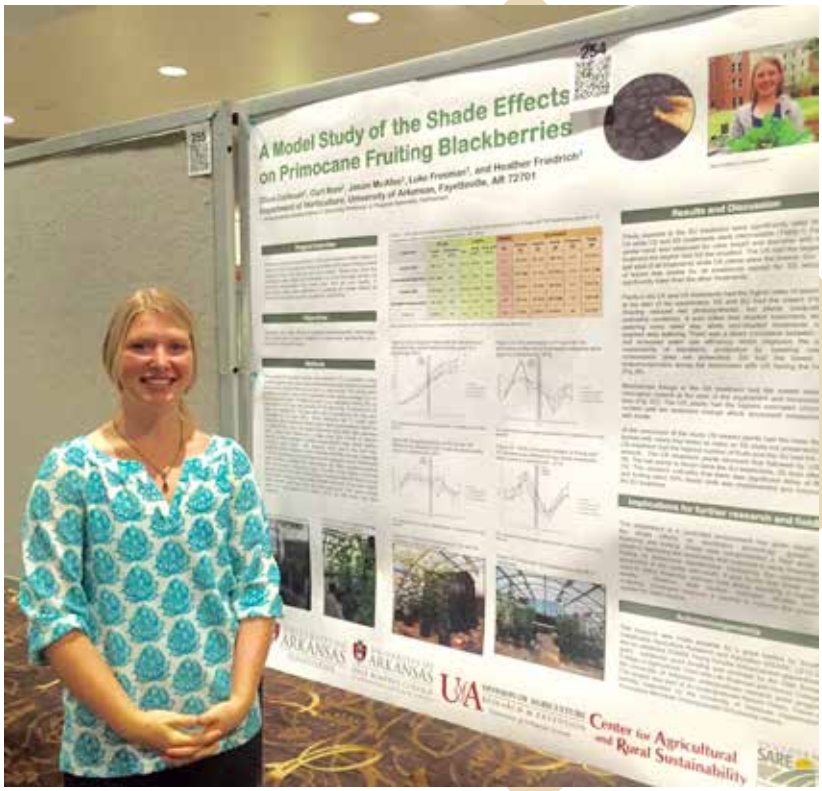
Bumpers College Students In Action



Olivia Caillouet. (**Horticulture). (left)** presenting her Greenhouse Experiment in the 2015 National American Society for Horticultural Science (ASHS) poster competition in New Orleans, Louisiana. Caillouet received 3rd place among undergraduates from across the nation. This travel was funded by Bumpers College, the Honors College Travel Grant, and Horticulture Department Michener Undergraduate Scholarship Award. **(below)** Olivia takes chlorophyll and gas exchange measurements of her blackberry plants**.** Her research on blackberries and internship at the Arkansas Agricultural Experiment Station, Fayetteville was made possible by a grant funded by Southern Sustainable Agriculture Research and Education (SSARE; LS12-250) and an additional S-SARE Young Scholar Enhancement apprenticeship grant.

Internships

Olivia Caillouet. (Horticulture). (Left) Olivia learned about bee-keeping during internships in both California and Puerto Rico. She desribes it as a life-changing experience, "I love the connection it shares with horticulture – it's such a symbiotic relationship. So much of the food we eat wouldn't be possible without pollinators." In this photo they are providing the weaker hives with supplemental sugar water. Olivia states that "this process is not ideal, however necessary in some instances to build the strength of hives prior to spring bloom and the honey production cycle of the year."

California

Puerto Rico

(above) Harvesting bananas in Puerto Rico at La Tierra Verde farm, which is privately owned by a family from Arkansas, for only 6 months at the time Olivia arrived. **(left)** Olivia harvests Meyer lemons at the 20 year old Country Flat Farm in Big Sur, California. With approval from Curt Rom, her honors thesis advisor, she interned there through the World Wide Opportunities on Organic Farms program for course credit at the organic orchard. She says of the two farms that they provided a great contrast between a farm just starting out and one that has been operating for decades. She also learned that she has "a passion for teaching and sharing my knowledge of ecologically sound farm practices with others."

Mitchell Pruitt. ((**Environmental, Soil, and Water Science).** The summer after graduation, Mitchell participated in in a conservation science internship at Hawk Mountain Sanctuary, the world's premier raptor research organization, near Orwigsburg, Pennsylvania. He was a field experience intern and was involved, mainly with their American Kestrel nest box monitoring program, but also with Turkey Vulture road surveys and with capturing/tagging Black Vultures. This was a wonderful experience that he says helped increase his knowledge in raptor conservation, as well as instill a desire for raptor research in his future career. Mitchell is pictured **(left)** with a newly tagged Black Vulture ready for release!

Pennsylvania

(Above and left) nestling American Kestrels (both males) that were two of over 100 individuals banded by Mitchell and colleagues this summer. The ladder is going up to the nest box.

Service Learning Experience Abroad

Paige Acklie. (**Agribusiness). (Left)** Paige cradles a chick during her travel abroad experience to Nampula, Mozambique through the Community Development in Mozambique summer program, which unites teams of poultry science, business and engineering students who tackle problems that range from assessing chick quality to corralling data from 33 different chicken farmers. During this hands-on service learning experience at New Horizons poultry farm Paige was part of the "Surveying Hunger" team which collected data surveying the diet of 60 families in the area, comparing the health of outgrowers to those not employed by New Horizons. **(below)** from left: Maggie Jo Hansen (center), translator Ibrahim Hamido and Paige Acklie conduct a dietary survey.

Mozambique

International Research Experience

Dylan Milholen (Environmental, Soil, and Water Science). Dylan participated in a CAFLS International Research Experience during the summer of 2015 in Brazil. He studied at UFPel in Pelotas, Rio Grande do Sul. **(Above) 3rd from right standing** ???. **(below)** He is pictured (4th from right) with graduate students standing in the research field on the UFPel farm after collecting grass samples to analyze their chemical compositions.

Field Research

Mitchell Pruitt. (**Environmental, Soil, and Water Science).** These photos were taken during two field seasons researching the occurrence of the Northern Saw-whet Owl in northwest Arkansas, a species previously not known to occur regularly in Arkansas. The first photo (top left) includes Mitchell with the first bird captured in fall 2014, proving the species' existence in the state during fall migration. **(below)** a bird in front of the banding setup. **(bottom)** a saw-whet being banded. Note the equipment: Pesola spring scale, aging diagrams, banding pliers, string of bands, field notes. All birds were captured at the Ozark Natural Science Center in Madison County, northeast of Fayetteville.

Oral and Poster Presentations at Conferences

Jack McCoy. (Horticulture). (Above) Pictured at the Southern Region American Society of Horticulture Science conference in San Antonio, February 2016, with his presentation entitled "Evaluation of Harvest Time/Temperature on Postharvest Incidence of Red Drupelet Reversion Developent and Firmness of Blackberry (*Rubus* L. subgenus *Rubus* Watson)". **(Below)** Pictured with his 2nd place award in the paper competition with his mentor, Distinguished Professor John Clark. For this same presentation, he also won 3rd place in the Gamma Sigma Delta undergraduate oral competition.

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- Use a comma before the word *and* in a series: *The U.S. flag is red, white, and blue.*

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The *Abstract* summarizes the purpose, procedures, and main findings in 250 words or less.

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The *Results and Discussion* section presents appropriate data, but not all data, in text, tables, and figures and places the findings in context with other research in the field. The discussion emphasizes new and important aspects of the research and conclusions that follow from them. Include implications and impact of the findings. Relate your findings to observations of other studies. State new hypotheses when warranted, but avoid unqualified statements not supported by your data.

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Acknowledgments

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