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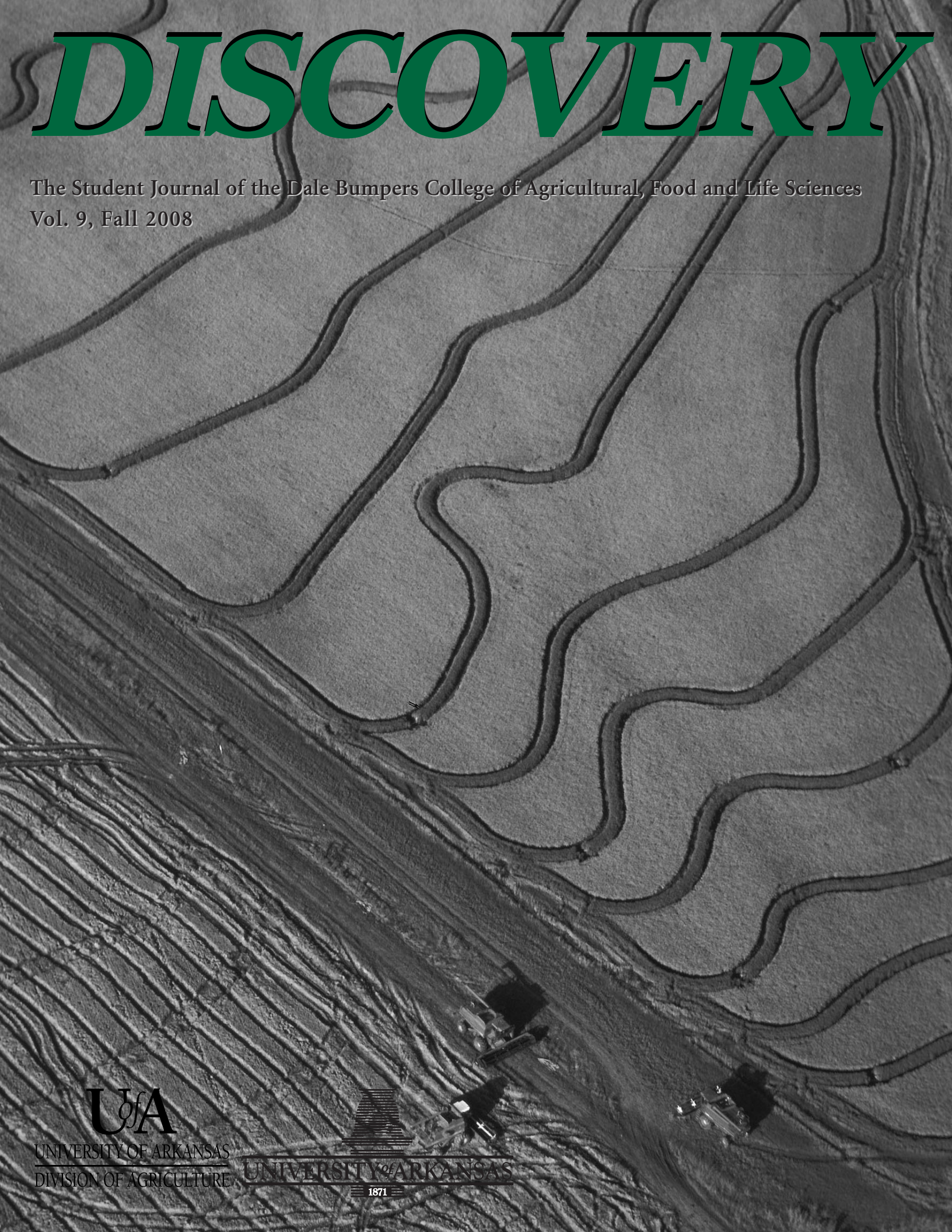

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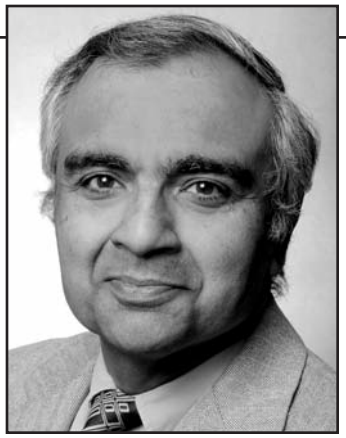
Cover: The Food and Agriculture Organization of the United Nations estimates that worldwide rice paddy production in 2008 may grow by about 2.3 percent for a new record level of 666 million tons. Yet rice prices will remain high in the face of input costs, natural disasters, and food shortages. Photo of US rice harvest by David Nance, USDA Agricultural Research Service.

Letter from the Dean



Lalit R. Verma

The Dale Bumpers College of Agricultural, Food and Life Sciences provides many opportunities for undergraduate students to engage in learning experiences beyond regular course work. Conducting quantitative/qualitative research projects with faculty mentors is one of those opportunities. Some sixty faculty members presently serve as mentors to students to assist them with research and creative projects. Most, but not all, such projects are designed to meet the requirements of an honors thesis in the Bumpers College Honors Program. Whether in the Honors Program or not, students who take advantage of such opportunities hone their intellectual capacities in a supportive, collaborative research environment that can lead to lifetime-long collegial, professional relationships.

The Bumpers College encourages student research by awarding undergraduate research grants, including the Carroll Walls Undergraduate Research Fellowship, which provides grants ranging from $500 to $2,000. We awarded 10 Undergraduate Research Grants in fall 2007.

DISCOVERY serves to showcase the research accomplishments of Bumpers College student scientists and allows them to participate fully in the process of shepherding their own research from project design through data collection to journal publication.

The 13 articles in this ninth annual volume of *DISCOVERY* include issues in food safety, nutraceuticals, rice, cover crops, animal husbandry, and forages. Topics also include environmental and quality-of-life issues such as watershed-based monitoring, phytoremediation of contaminated soils, and community-based gerontology research. We are proud to present these articles as examples of the research accomplishments of our undergraduate students.

I congratulate the student authors on their accomplishments and extend thanks to their faculty mentors and to the editors who reviewed their manuscripts. The UA Division of Agriculture, University of Arkansas Agricultural Experiment Station commendably provides funding for research, and Dr. Milo J. Shult, Vice President for Agriculture, generously supports students who participate in various competitions in their disciplines. Thanks are also extended to the Honors Committee for providing a structured program that encourages our students to enhance their academic and professional credentials.

Lalit R. Verma, Interim Dean and
Associate Vice President–Academic Programs

Life cycle analysis for the cultivation and combustion of miscanthus for biofuel compared with natural gas

Amanda Ashworth[*], *Charles West*[†], *Michael Popp*[§], *Mireille Montrejaud-Vignoles*[‡], *Caroline Sablayrolles*[§§], *and Benoît Gabrielle*[‡‡]

ABSTRACT

As negative environmental and economic impacts of fossil fuels have escalated, so has the importance of renewable bioenergy crops whose feedstocks are noncompetitive with food supplies. Compared with fossil fuels, use of lignocellulosic feedstocks offers potential for greenhouse gas reduction and highly positive net energy returns because of low input demand and high yields per unit of land area, thus making them advantageous for the emerging biofuel industry. The aim of this study was to simulate environmental impacts of producing a biofuel grass for combustion use based on the inventory of inputs and their effects on eutrophication of surface waters; acidification of land and water; photochemical ozone-creation potential (i.e. smog); global atmospheric warming; and nonrenewable resource depletion (mainly fossil fuels). Hybrid miscanthus (*Miscanthus* x *giganteus*, or giant miscanthus), a perennial C4 grass originating from East Asia, was compared with natural gas by using a life-cycle analysis model for biomass production in France. The analysis showed a trade-off between natural gas and miscanthus. The latter had a lower global-warming potential and consumed less primary nonrenewable energy but produced more emissions that promote acidification and eutrophication than did natural gas.

[*] Amanda Ashworth graduated in August 2008 with a B.S. in environmental soil and water science.

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[§] Michael Popp is a professor in the Department of Agricultural Economics and Agribusiness.

[‡] Mireille Montrejaud-Vignoles is a professor at the Ecole Nationale Supérieure des Ingénieurs en Arts Chimiques et Technologiques, Toulouse, France.

[§§] Caroline Sablayrolles is a professor at the Ecole Nationale Supérieure des Ingénieurs en Arts Chimiques et Technologiques, Toulouse, France.

[‡‡] Gabrielle Benoît is a researcher at the Institut National de la Recherche Agronomique, Thiverval-Grignon, France.

MEET THE STUDENT-AUTHOR

Amanda Ashworth

I am from Fayetteville, Ark., and a graduate of Fayetteville High School. At present, I am a graduating senior at the University of Arkansas majoring in Environmental, Soil, and Water Science with minors in Spanish and Global Agriculture, Food, and Life Sciences. I am a member of the Crop, Soil, and Environmental Sciences Club.

During my career at the University of Arkansas, I spent a year studying at the Universidad de Granada to complete my Spanish minor. I also received two Americorp Education Awards through my completion of two Student Conservation Association (SCA) internships. One internship included conserving native-plant populations in Montana with the USDA Forest Service, and the second internship was with the Bureau of Land Management in California working on safeguarding dry-land species against extinction. Since last May, I have worked in the Crop, Soil, and Environmental Sciences Department as a lab and greenhouse assistant on native-plant propagation for wetland/riparian restoration and biomass production.

The research project reported in this paper was made possible by the Renewable Resources and Clean Technology International Program, and through partnership of the National Polytechnic Institute of Toulouse, France, and the University of Arkansas. A poster presentation of this study was presented at the International Conference on Renewable Resources and Biorefineries in The Netherlands in June 2008.

I would like to give special thanks to Drs. Charles West and Michael Popp of the University of Arkansas for their advice on this project. Sincere appreciation is extended to Caroline Sablayrolles and Mireille Montrejaud-Vignoles for hosting my spring semester at the Ecole Nationale Supérieure des Ingénieurs en Arts Chimiques et Technologiques (ENSIACET), and for the expert knowledge and guidance they devoted to this project.

I started my master's degree program in the Crop, Soil, and Environmental Sciences Department in August 2008, with Dr. West as my advisor. My graduate research plans include continuing this study using bioethanol as the endpoint, and researching switchgrass biomass and nutrient accumulation in Arkansas.

INTRODUCTION

Life-cycle analysis (LCA) is a cradle-to-grave environmental diagnostic tool that calculates energy and material inputs and outputs of potential pollutants at every stage of fuel production and consumption. Such analyses are critical for comparing alternatives to fossil fuels to maximize energy efficiency and minimize environmental degradation. Replacing fossil fuels with plant-derived feedstocks causes a decrease in net carbon emissions because of withdrawal of carbon from the atmosphere during photosynthesis, thereby theoretically reducing net greenhouse gas (GHG) emissions. Little is known, however, about the magnitude of potentially negative environmental impacts and trade-offs in harnessing the sun's energy through combusting plant biomass. Life-cycle analyses are also useful to determine the most efficient practices for biomass production, transport, storage, and processing in terms of being least cost and least detrimental to the environment (Schmer et al., 2008), and thus have relevance for policy making and industrial-scale design of bioenergy systems.

This LCA aims to quantify the nature and magnitude of pollution trade-offs when analyzing the perennial grass, hybrid miscanthus (*Miscanthus* x *giganteus*, or giant miscanthus), as an alternative to natural gas. Miscanthus is classified as lignocellulosic biomass feedstock because the entire plant is used. Most of the gross energy is contained in the fibrous (lignin, cellulose, and hemicellulose) component of the plant. Such a feedstock

can be used directly for heat and electrical power generation through simple combustion, which aids in reducing greenhouse gas emissions through direct replacement of fossil fuels. In comparison, production of corn (*Zea mays* L.) for bioethanol is currently the main source for ethanol production in the U.S.; however, corn provides minimal net benefits in terms of reducing fossil energy consumption and GHG emissions (Tillman et al., 2006).

Miscanthus was chosen for this study because it produces high biomass yields with low levels of industrial inputs, such as fertilizer, pesticides, and irrigation (Clifton-Brown et al., 2004), when compared with annual crops like corn. In contrast, annual crop production destabilizes soil through repeated cycles of soil cultivation, crop establishment, and harvest, which lead to higher erosion than perennial crops (Lewandowski and Schmidt, 2006). Perennial crops such as miscanthus have the added advantage of not requiring annual tillage and planting operations, which further reduces energy inputs and negative environmental impacts. We hypothesized that miscanthus production for use in heat and power generation results in a lower release of GHG and ozone-creating compounds that induce smog, but a greater release of compounds inducing eutrophication and acidification when compared with fossil fuels such as natural gas.

MATERIALS AND METHODS

Four phases of this analysis included 1) goal and scope definition, 2) life cycle inventory, 3) life cycle inventory assessment, and 4) interpretation of data. Figure 1 illustrates the production and collection stages for the two fuels in this comparison. The analysis included all the agricultural processes involved in producing biomass and subsequent direct combustion, such as stand establishment, application of fertilizers, machinery for transportation and harvest, and pesticides as well as estimating GHG emitted during these processes. A range of environmental parameters were analyzed and aggregated into the following impact categories: resource depletion (comprising primary, mainly fossil, energy consumption to supply electricity and buildings, machinery, chemicals, etc.); acidification; eutrophication; creation of photochemical ozone (smog) via nitrous oxide emission (e.g. depletion of the protective stratospheric ozone); and greenhouse gas emissions for calculating global warming potential (GWP) with a time horizon of 500 years (Table 1). Global warming potential is an indicator of the heat retention capacity of a gas to impact climate. This LCA does not include any economic or social functions, nor does it calculate net energy yield or net energy ratio of biofuel production systems. Renewable energies that contribute to the primary energy pool and other indirect energies that contribute to crop production, such as human labor, are considered as outside the system.

We used the LCA methods and input/output outlined by Institut für Energie und Umweltforschung (IFEU) (Institute of Energy and Environmental Research Heidelberg, 2000) and the pollution standards of the Association Française de Normalisation (2006a, 2006b). The database for calculating the LCA was described by Gabrielle et al. (2001). The fuel use and production are expressed as megajoules (MJ) per hectare, and emissions (environmental impacts) as grams (g) of emission equivalents per hectare.

The values for miscanthus management and yield were applicable to France using data and default values from the Institut National de la Recherche Agronomique (INRA) (Gabrielle et al., 2001). We assumed standard agricultural inputs and practices including the use of typical machinery for field preparation, planting, harvest, and transportation, from which we calculated the corresponding emissions using the IFEU standards. Miscanthus plants were presumed to have a useful stand life of sixteen years. Establishment requires two years before the first harvest, followed by a single harvest annually yielding 25 metric tons per hectare (ha). Weed control was required only in the first year, and fertilizing started in the second year at 50 kg per ha of N. Phosphorus and potassium were not added because soil levels were assumed to be adequate, and plant uptake and removal were very low (Lewandowski and Kircherer, 1996). The harvest method was chopping for loose hauling, presuming a loss of plant dry matter of 5%. This compares with 10-30% loss from the round-baling method (ADEME, 1998). Ash disposal to a landfill after combustion was also considered as a byproduct. The environmental impacts of the agricultural production processes were averaged over the lifetime of the crop to obtain annual values. Economic evaluations, not conducted to date, will use discounting.

The fossil fuel life-cycle analysis was carried out similarly to the miscanthus LCA by taking into account all processes involved in resource extraction, processing, and utilization. Natural gas production entailed extraction, transportation, compression, processing, and finally distribution to the consumer. Natural gas was assumed to be extracted in Norway and Russia and distributed throughout France. The crude oil was extracted in OPEC countries and transported to Europe with transport costs calculated using average distances (IFEU, 2000). These choices are based on expert opinion and current technology (IFEU, 2000). The IFEU report provided an assessment of the relative reliability of environ-

mental impacts. Since empirical data were very limited or nonexistent for miscanthus, values were extrapolated from other crop production systems and qualified by in-country experience (Benoît et al., 2001). We decided to analyze only those environmental impacts whose estimates and data sources were considered by IFEU (2000) to be reliable. Impacts excluded from this analysis included stratospheric ozone depletion, human toxicity (e.g. carcinogens, heavy metals, particulates), and ecotoxicity agents (e.g. heavy metals and recalcitrant organics).

For the life-cycle inventory assessment, sums of impacts for all processes were converted into functional units of MJ/ha or g emissions/ha to calculate total impacts for the entire production chain. We subtracted these sums from the reference system for miscanthus. A normalization step, or ranking, was carried out to compare the results over a range of variables and impact categories, including conversion to percentage of total impacts to simplify the presentation and interpretation of data. Calculations and graphing were carried out using Microsoft Excel® spreadsheets.

RESULTS AND DISCUSSION

Standardized outputs of primary energy depletion and environmental pollutants for simulated miscanthus production are illustrated in Fig. 2. All impacts were small in comparison to global warming potential resulting from fertilization, harvest, and transport. Fertilization impact is relatively large because the process of converting atmospheric nitrogen gas (N2) into ammonia is energy intensive and consumes natural gas as a source of hydrogen. Thus, any energy-efficient, plant-derived biofuel system must be one which has very low nitrogen fertilizer requirements. Miscanthus is a crop which is relatively efficient in nitrogen use and conversion to biomass yield (Lewandowski and Schmidt, 2006). Harvest and transport also consume significant amounts of fossil fuel and thus emit measurable amounts of GHG, suggesting the importance of developing improved methods of handling bulky feedstocks such as plant biomass.

The wide range of orders of magnitude of the output values necessitates comparing the two fuels on a percentage basis to more easily visualize their relative impacts. Figure 3 illustrates relative contributions to each impact category of each production process, ranging from seedstock production to ash disposal. The entire value of an impact, be it in MJ of primary energy depletion or g of emissions, is represented by 100%. Combustion contributed the large majority of emissions resulting in ozone creation (photochemical ozone creation potential, POCP, or smog), eutrophication, acidification, global warming potential, and primary energy depletion. The latter category essentially represents fossil-fuel depletion involved in all nonrenewable energy consumption processes. Eutrophication, acidification, and ozone creation are explained by release of nitrogenous, sulfurous, and phosphatic compounds to the soil, water, and atmosphere resulting from fertilization of the crop and from combustion and release of gases (Table 1). Harvest and biomass transport impacted the environment less adversely than fertilization (Fig. 3), whereas seedstock production, field preparation, planting, and pest control contributed negligible amounts to environmental impacts.

Values for comparing miscanthus vs. natural gas are summarized in Table 2. Natural gas had substantially greater resource depletion (3.6-fold) and global warming potential (2.0-fold) than miscanthus. Photochemical ozone creation potential was essentially the same between the two energy sources. In contrast, acidification and eutrophication impact values were lower and thus more favorable for natural gas than miscanthus, based on our analyzed system. The calculated differences between the fuel types indicate which had a more favorable environmental impact. The negative values represent an advantage for the bioenergy when compared to its fossil fuel counterpart. Likewise, positive values show a disadvantage for the biofuel. Results are also presented as relative percentages of the sum of the two energy types (Fig. 4). This presentation normalizes the data and places the impact categories on the same scale. The advantage of miscanthus over natural gas in reducing nonrenewable resource depletion and global warming potential is again clear, as is the relative advantage of natural gas in reducing acidification and eutrophication.

It is clear that replacing a nonrenewable fossil fuel such as natural gas with a renewable, perennial biofuel crop would greatly reduce depletion of fossil fuel reserves, even though some fossil energy consumption occurs with production, harvest, and transport of the crop. The annual photosynthetic ability of miscanthus greatly reduces net CO_2 emissions and thus reduces GHGs and the global warming potential. Lewandowski et al. (1995) concluded that combusting 20 metric tons/ha miscanthus emits a net 2.2 tons CO_2, whereas combusting the same energy equivalent of hard coal emits 34 tons CO_2. Therefore each hectare of miscanthus would directly reduce emission of 31 tons CO_2 per year (90% reduction) when compared with hard coal. In addition to CO_2, emissions include other GHGs such as CO, CH_4, and N_2O (Kaltschmitt et al., 1997). Use of low-net-emission biofuels combined with minimal fossil energy consumption during conversion would have more favorable effects on atmospheric conditions, particularly global warming reduction, than any fossil fuel.

The disadvantage of miscanthus in terms of acidification and eutrophication demonstrates that biofuel crops are not completely benign in their potential environmental impact when used in combustion. Sources of emissions in these categories are mainly from the combustion process itself (Fig. 3), which oxidizes organic S and N in plant biomass to SO_2, NO_x, and other trace compounds, which convert to acids in the atmospheric water and return to soil and surface waters as precipitation or dry fallout. Natural gas is a relatively clean-burning fossil fuel, especially in relation to coal. Soil acidification from nitrogen fertilizer was assumed to occur in this LCA, and fertilization of the biofuel crop to produce high yields results in some degree of leakage of nutrients off-site. The ability of miscanthus to retain and internally recycle environmentally sensitive macronutrients such as N, P, and S is poorly understood. Efficient nutrient recycling of such nutrients would be expected to minimize the eutrophication impact of producing perennial biofuel crops.

Conclusions

We conclude that the lignocellulosic feedstock, miscanthus, is a more environmentally beneficial fuel source than natural gas in terms of global warming potential when comparing their use for combustion for district heating. Miscanthus production would theoretically involve zero net carbon emissions when only considering the re-assimilation of CO_2 via photosynthesis that had been previously emitted through combustion; however, use of fossil fuels in nitrogen fertilizer synthesis, delivery, and application and the harvest and transport of biomass consume some fossil energy. Site conditions, nitrogen fertilizer-use efficiencies by different feedstocks, and local economic factors must be taken into account when selecting a fuel source that will create the most environmentally benign system. The agronomic properties of miscanthus make it a promising plant species for bioenergy in France and potentially the U.S. because it produces high biomass yields with a low level of industrial inputs, such as fertilizers and pesticides. The favorable CO_2 balance of this feedstock emphasizes its efficiency as a fuel source, especially considering current global climate change. It is important to note that other biomass species, such as switchgrass (*Panicum virgatum*), may lead to different results if our assumptions do not apply. The comparison of biogenic and fossil fuels shows clear advantages and disadvantages with both fuel options, and decision-makers must consider the trade-offs based on the acceptance of the various ecological impacts on a worldwide basis.

Further research should include field trials and comparative analyses with other biofuel feedstocks in multiple sites in Europe and the U.S. to more accurately quantify the net energy balances and environmental impacts than just those estimated in this simulation model. Life-cycle analyses are useful complements to field trials to estimate environmental advantages of alternative biofeedstocks that could replace nonrenewable fossil fuels.

ACKNOWLEDGMENTS

The support of the Dale Bumpers College of Agricultural, Food and Life Sciences and the University of Arkansas Division of Agriculture is gratefully acknowledged.

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Table 1. Impact classification of pollutants indexed in the life cycle inventory.

Impact categories	Pollutants inventory
Primary energy depletion	Primary energy inputs: natural gas, petroleum, coal, and uranium
Global warming potential (GWP)	Volatile organic compounds (VOCs) CO_2, CO N_2O, CH_4
Ozone depletion	N_2O
Photochemical ozone creation potential (POCP, smog)	Benzene (C_6H_6), Methane (CH_4), VOCs CO, Hexane
Acidification	NH_3, HCl, NO_x, SO_2
Eutrophication	NO_x, NO_3^-, NH_3, NH_4^+, PO_4^{-3}

Table 2. Resource depletion and emission values for miscanthus and natural gas. Negative values for the difference between the energy types indicate an environmental benefit from using the bioenergy crop over the fossil fuel.

Environmental impact	Balance parameter	Unit (per hectare, per year, per MJ of heat)	Bioenergy life cycle (miscanthus)	Fossil fuel life cycle (natural gas)	Difference (bioenergy-fossil fuel)
Resource depletion	Primary energy	MJ	0.3415	1.2336	-0.8921
GWP500	CO_2 equivalents	g	35.128	69.814	-34.685
POCP	Ethylene equivalents	g	0.0182	0.0199	-0.0017
Acidification	SO_2 equivalents	g	0.2910	0.0601	0.23090
Eutrophication	NO_3 equivalents	g	0.3092	0.0785	0.2306

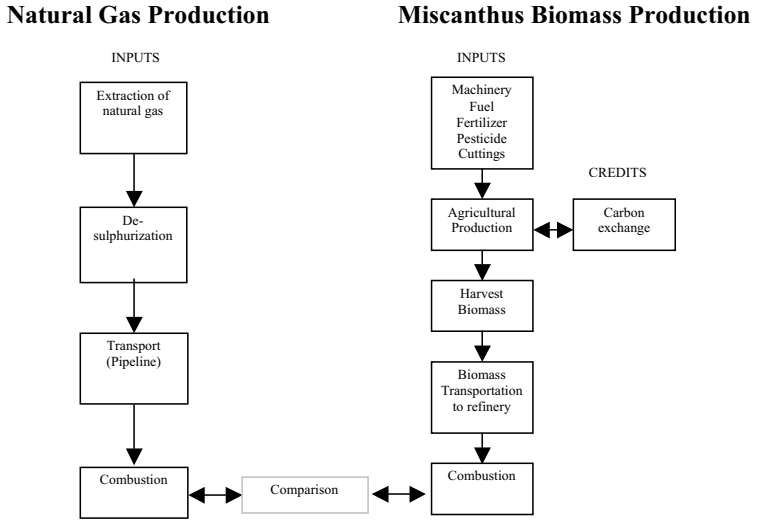

Fig. 1. Standard life cycle comparison of natural gas and miscanthus production

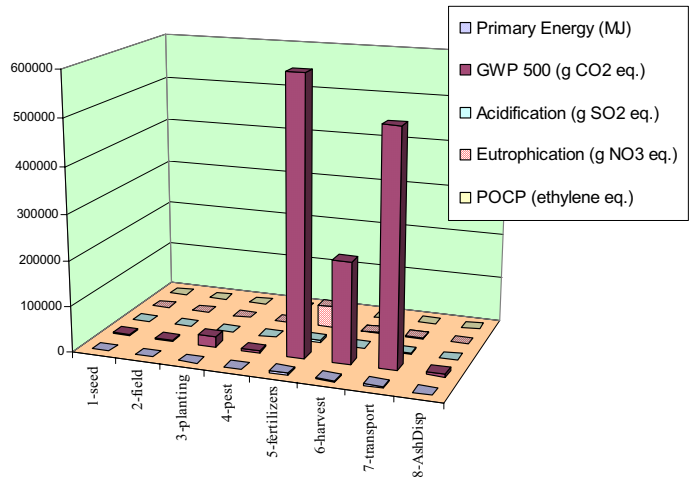

Fig. 2. Impacts of miscanthus production steps on environmental impact categories expressed as standardized functional units, megajoules (MJ) or grams (g) per hectare, as appropriate.

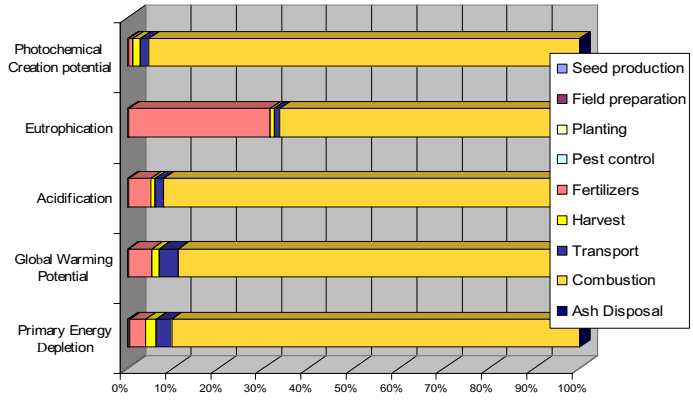

Fig. 3. Relative contributions of miscanthus biofuel production and combustion processes to each environmental impact classification.

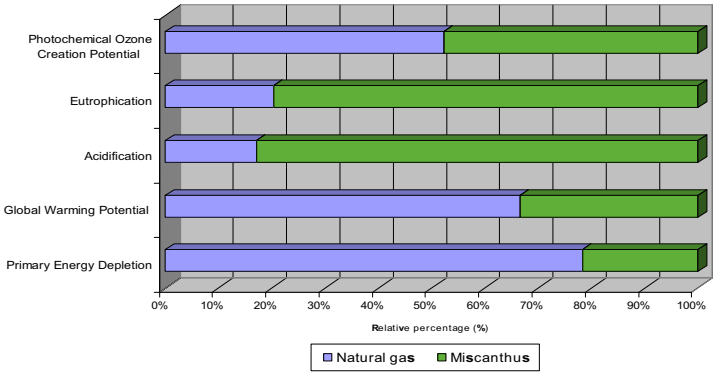

FIG. 4. Comparisons of miscanthus vs. natural gas for their environmental impacts. Fuel type with a horizontal bar greater than 50% indicates more negative environmental impact.

An acreage response model for Arkansas rice farms

J. Grant Ballard[*] *and Michael R. Thomsen*[†]

ABSTRACT

In recent years, market forces have signaled a strong demand for rice as well as other Arkansas crops. However, high fuel, fertilizer, and chemical costs have negatively impacted farm income, and these input costs are widely known to impact planting decisions of farmers. The goal of this study is to develop and estimate an acreage response model for rice. The model is used to compute acreage response elasticities and provides insight into roles that input costs and crop prices play in acreage decisions made by producers. Economic theory predicts that prices for important inputs such as fuels and fertilizers as well as the relative prices of rice and soybeans will impact acreage decisions. Soybean prices are expected to be important because most of the machinery needed to produce rice and soybeans is the same and these crops are already used commonly in rotation. Results of the study show that crop price variables do indeed play a significant role in producer planning. Short- and long-run own-price acreage response elasticities are estimated to be 0.69 and 1.19, respectively. Soybean prices have the expected negative impact on rice acreage with a cross-price elasticity of -0.33 in the short run and -0.57 in the long run. On the other hand, the expected economic impacts of input prices on rice acreage were not supported by the results. Estimated relationships were negative, as would be predicted by economic theory, but were not statistically significant.

[*] J. Grant Ballard completed his BSA in agricultural business in December 2007 and is entering the University of Arkansas School of Law in August 2008. This paper is based on his honors research project that he completed while a senior in the Dale Bumpers College studying in the Department of Agricultural Economics and Agribusiness.

[†] Michael R. Thomsen in an associate professor in the Department of Agricultural Economics and Agribusiness.

MEET THE STUDENT-AUTHOR

J. Grant Ballard

I am a native of Roland, Ark., and a 2004 graduate of Little Rock Central High School. Upon graduation from high school, I enrolled at the University of Arkansas in the Department of Agricultural Economics and Agribusiness. I completed my B.S. degree in December 2007 with a major in agribusiness and a minor in environmental, soil, and water science. While an undergraduate at the University of Arkansas, I was a member of the Alpha Gamma Rho Fraternity, Agribusiness Club, Collegiate Farm Bureau, Order of Omega, and Alpha Zeta. I also had the honor of serving the Bumpers College as a student ambassador. I was Vice Noble Ruler of Alpha Gamma Rho in 2006, and I was president of the University of Arkansas chapter of Collegiate Farm Bureau in 2007. I will begin law school in fall 2008 at the University of Arkansas at Fayetteville.

This field of research was selected because of my personal interest in agricultural economics and rice production as well as the economic importance of rice production and milling to the study region and the state. I would like to thank Dr. Michael Thomsen for his guidance, time, and commitment to this study.

INTRODUCTION

In recent years, market forces have signaled a strong demand for rice and other Arkansas crops. This demand fueled a growth in Arkansas crop sales. However, high fuel, fertilizer, and chemical costs have negatively impacted farm income (Childs and Livezey, 2006), and these input costs are widely known to impact planting decisions of farmers. In eastern Arkansas, rice and soybeans are commonly grown in rotation in order to control weed populations in rice fields. Most eastern Arkansas soils that are suited for rice are also well suited for soybean production, and that makes this arrangement economically sound. Growers clearly have the option to grow rice, soybeans, or other possible crops. Aside from agronomic considerations, producers will base their planting decisions on expected profitability (Kay, Edwards, and Duffy, 2004). For a variety of reasons, a producer could decide to dedicate more of his/her acreage to soybeans or other crops and less to rice. Rice is more capital intensive than soybeans (Childs and Livezey, 2006), and in years with higher input costs or lower average rice prices, it would be expected that a lower than average acreage would be planted in rice.

The objective of this study is to develop and estimate an acreage response model for rice. The model will be used to compute acreage response elasticities and will provide insight on the role that input costs and crop prices play in acreage decisions made by producers in the selected study region. It is expected that prices for important inputs such as fuels and fertilizers as well as relative prices of rice and soybeans will impact acreage decisions. Soybean prices are expected to be important because most machinery needed to produce rice and soybeans is the same and these two crops are already used commonly in rotation.

MATERIALS AND METHODS

Acreage Response Model

Shumway (1986) summarizes supply relationships for several southern US crops and provides an overview of empirical methods of supply estimation. Economic theory indicates that supply depends on the price the producer expects to receive at harvest along with the prices of inputs and the expected profitability of other competing crops (Hudson, 2007). Several approaches are available for specifying an expected price. Nerlove (1958) championed the use of the adaptive expectations model for the analysis of agricultural supply functions. This is a very common approach and many textbooks on

the subject of econometrics discuss this model and present issues involved in its estimation (Maddala, 1992; Kmenta, 1986). The adaptive expectations model provides one way to address price expectations even though such expectations are not observed. Producers cannot always accurately predict price they will receive but it is reasonable to assume that producers' expectations of price are expressed as a weighted average of past prices (Nerlove, 1958). The weights of past prices are functions of λ, where λ is a coefficient between zero and one. Specifically, expected prices are expressed as:
(1)

$$P^*_{t+1} = (1-\lambda)P_t + (1-\lambda)\lambda P_{t-1} + (1-\lambda)\lambda^2 P_{t-2} + (1-\lambda)\lambda^3 P_{t-3} + \ldots\ldots$$

According to equation (1) the expected price in any given period depends on prices that have been observed in the past. The influence of observed prices on this expectation will decline as one goes back in time, since λ is between zero and one. The goal is to represent expected price in some manner that does not require an infinite number of observations on past prices. This can be accomplished by lagging equation 1 by one period and multiplying through by λ to get:
(2)

$$\lambda P^*_t = (1-\lambda)\lambda P_{t-1} + (1-\lambda)\lambda^2 P_{t-2} + (1-\lambda)\lambda^3 P_{t-3} + \ldots\ldots\ldots$$

Subtracting equation (2) from equation (1) provides:
(3)

$$P^*_{t+1} - \lambda P^*_t = (1-\lambda)P_t$$

The usefulness of equation (3) will be clear momentarily.

A linear econometric model for the acreage response function for rice is given by:
(4)

$$Q_t = \alpha + \beta P^*_t + \sum_{i=1}^{N} \gamma_i Z_{it} + U_t$$

Where Q_t is acreage, P^*_t is expected rice price, and the Z_{it} are exogenous variables reflecting the profitability of soybeans (a competing crop) and prices of inputs. Lagging equation 4 by one period and multiplying through by the partial adjustment coefficient, λ, provides:
(5)

$$\lambda Q_{t-1} = \lambda\alpha + \lambda\beta P^*_{t-1} + \lambda \sum_{i=1}^{N} \gamma_i Z_{it-1} + \lambda U_{t-1}$$

Subtracting equation 5 from equation 4 provides:
(6)

$$Q_t = (1-\lambda)\alpha + \lambda Q_{t-1} + \beta(P^*_t - \lambda P^*_{t-1}) + \sum_{i=1}^{N} \gamma_i (Z_{it} - \lambda Z_{it-1}) + U_t - \lambda U_{t-1}$$

which, by equation 3, can be expressed in terms of observed variables as:
(7)

$$Q_t = (1-\lambda)\alpha + \lambda Q_{t-1} + \beta(1-\lambda)P_{t-1} + \sum_{i=1}^{N} \gamma_i (Z_{it} - \lambda Z_{it-1}) + U_t - \lambda U_{t-1}$$

Equation 7 is a function of observed variables and can be used to uncover estimates of the parameters from the theoretical model in equation 4. There is one problem that complicates the estimation of equation 7 in that the error term is correlated with Q_{t-1}, one of the regressors. As a result, the method of ordinary least squares will provide inconsistent parameter estimates. For this reason, Maddala (1992) discusses the use of non-linear least squares to estimate equation 7.

Computation of Elasticities

The parameter estimates observed from equation 7 make it possible to calculate point estimates for acreage response elasticities. The estimated own-price coefficient (β) and estimates for coefficients on exogenous variables (γ_i) are used to determine point estimates for acreage response elasticities. Specifically, the own-price elasticity of supply is given as:
(8)

$$\beta \frac{P}{Q}$$

and the elasticity of supply with respect to the ith exogenous variable is given by:
(9)

$$\gamma_i \frac{Z_i}{Q}$$

Long-run acreage response elasticities can be computed by dividing the short-run elasticity computed from either equation 8 or 9 by (1–λ) (Nerlove and Addison, 1958).

Data

Data for this study were collected from the United States Department of Agriculture National Agricultural Statistics Service (NASS, 2007) and the United States Department of Labor Bureau of Labor Statistics (BLS) (2007a; 2007b). NASS provided average yearly prices for rice and soybeans as well as county-level information on acres planted, acres harvested, and average yield. Producer Price indexes (PPI's) for petroleum products and for fertilizer products were collected from BLS (2007a) in order to demonstrate changes in prices paid

by producers for inputs. The producer price index measures average change over time in selling prices received by domestic producers for their output. The BLS's (2007b) Consumer Price Index (CPI), which measures general inflation in the US economy, was used to adjust all prices and PPI measures for inflation.

The study region consists of 55 counties and parishes in the states of Arkansas, Mississippi, Missouri, and Louisiana. The selected counties and parishes are all found in what NASS classifies as the Mississippi Delta region and in the East Arkansas Non-Delta region (Livezey and Foreman, 2004). Production practices throughout the region are similar, and costs of production as well as returns should be fairly standard across the region. In order to accurately assess impacts of input costs and crop prices upon acreage decisions of farmers, only those counties with a regular history of rice production were included. The criterion used to include or exclude counties from the study region was for the county to have reported rice production in each of the past 10 years. A graphic of the study region used for this study is presented in Figure 1.

Rice acreage, the dependent variable for the acreage response model, was obtained by summing acres harvested over all counties and parishes in the study region for each year. Based on 2000 to 2006 production data from NASS, the 55 counties and parishes in the study region accounted for 62% of all US rice production and approximately 80% of US long grain rice production. Soybean price was considered exogenous for the purposes of this study. In terms of national production, the study region was responsible for a small portion (approximately six percent) of the total US soybean production. The implicit assumption here was that producers in the study region can sell all the soybeans they want at prevailing prices.

In developing the dataset it was important to account for the impact of policy on producer behavior. Beckman (2005) provides a good summary of US farm policy that affects rice production. Supply controls such as acreage adjustments, marketing quotas, price supports, and storage of excessive supplies under loan were a part of the government policy toward basic US commodities for much of the twentieth century. The rice acreage allotment system was eliminated with the Farm Bill of 1981. The Marketing Loan Program allows producers to obtain a loan from the Commodity Credit Corporation (CCC). The producer uses his current year production as collateral. The loan rate creates a price floor, and producers are eligible for a marketing loan gain (MLG) when world price falls below the loan rate. Loan Deficiency Payments (LDPs) are also available to producers under the Marketing Loan Program. Again, these payments are available when world price falls below the loan rate. Producers can take this direct payment instead of securing a Marketing Assistance loan. Because the loan rate acts as a price floor that maintains a certain level of production, the price of rice was modified to reflect the larger of (a) the loan rate or (b) the price reported by NASS (2007). Loan rate data were gathered from the USDA's Economic Research Service.

The period chosen for this study reflects 30 years beginning with 1977 and extending through 2006. Although the earliest years in the dataset reflect conditions under the acreage allotment system, which limited producer responses to price signals, the goal here is to obtain a dataset that reasonably reflected recent production practices but still contained enough observations for the statistical methods to be reasonably powerful. Descriptive statistics for variables used in the acreage response model are reported in Table 1.

RESULTS AND DISCUSSION

Table 2 presents results of the estimated acreage-response model. The estimate column displays the parameter estimates of the model. These estimates demonstrate the impact of an increase in rice price, soybean price, fuel price, and fertilizer price. These estimated impacts can be used to determine how producers respond to changes in these variables. For example, if rice price were to increase by $1 per cwt., then according to the results rice acres harvested would increase by 173,643 acres. If soybean price was to increase by $1 a bushel, then rice acreage would drop by 108,915 acres. Continuing with this analysis, if fuel price and fertilizer price were to increase by the same amount per unit, acreage would shift down 125 acres and down 5,107 acres, respectively. However, one must also consider the magnitude of the t-values reported in Table 2. The t-values for fuel and fertilizer prices are small in magnitude and indicate a lack of statistical evidence for these variables having an effect on acreage decisions.

The conclusion to be drawn from Table 2 is that rice and soybean prices have the most significant impact upon acreage devoted to rice while fuel and fertilizer prices seem insignificant. The insignificant impacts of fuel and fertilizer prices are perplexing because economic theory would suggest acreage should be reduced as input prices increase. To explore the issue further the model was re-estimated without the fertilizer price because fuel and fertilizer prices are highly correlated. This alternative specification did not meaningfully change the results. After fertilizer was dropped, the fuel coefficient was still positive and insignificant.

Acreage response elasticities (Table 3) are computed

at the sample means. Again, it is important to keep in mind that the coefficients on fuel and fertilizer price variables were insignificant and so the fuel and fertilizer elasticities are suspect. The short-run own-price elasticity is 0.69 and can be interpreted as indicating that all else equal, a one percent increase in the price of rice will result in 0.69 percent increase in acreage devoted to rice production. The long-run own-price elasticity of 1.19 is larger in magnitude and is consistent with the idea that production decisions are more flexible over a longer planning horizon. Other elasticity estimates reported in Table 3 can be interpreted in the same fashion.

Discussion

The objective of this study was to determine acreage response of rice to changes in crop price and increasing costs of inputs. The goal was to identify the impact of these factors upon planting decisions of rice producers in the study region. Economic theory predicts that expected returns motivate planting decisions, and results presented here provide clear evidence that crop price variables play an important role in producer planning. All else equal, when price of a crop increases, supply will follow. Similarly, the model showed that as soybean prices increase, acreage shifts out of rice. This is again a basic economic principle that makes good sense for business application.

The expected economic impacts of input prices, on the other hand, were not supported by the results. Rice acreage was not found to be significantly responsive to fuel costs or fertilizer costs. This may suggest that input costs are not as significant as expected price when producers make their planting decisions. However, it is more likely that the lack of significance is a feature of the problem being analyzed. Specifically, inputs such as fuel make up about the same percentage of total cost for production with irrigated soybeans as with rice. Fuel costs average approximately 15 to 20 percent of cost for production with both crops (Watkins, 2006). Producers will spend less to make a crop of soybeans because the crop is not as input-intensive, but the returns will generally be higher for rice if the price is competitive due to rice being a higher yielding crop than soybeans.

Agriculture is changing rapidly in the present day. The 2006 crop year was the last year analyzed in this study. Since then, many new issues have arisen that could bring attention to the questions addressed in this study. Basis has reached record highs in many regions and the ethanol boom has shifted much acreage into corn and out of soybeans in the past year. In future research, a market equilibrium model could be useful for a study of the Midwest corn and soybean crops. In the Midwest, where corn and soybean prices would not be considered exogenous, it may be easier to estimate the impact that fuel prices have on equilibrium prices of these crops and then to examine how the impact of those price changes indirectly impact rice prices and consequently rice acreage.

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Table 1. Descriptive statistics for the study region 1977-2006.[A]

Variable	Description	N	Mean	Std Dev	Minimum	Maximum
Rharv	Rice Acres Harvested	30	1,694,145	316,640	985,490	2,227,400
Rprice	Rice Price ($/cwt.)	30	6.88	3.46	3.61	15.66
Sprice	Soybean Price ($/bu.)	30	4.99	2.18	2.47	10.21
Fert	Fertilizer Price (PPI)	30	81.05	15.41	57.70	111.33
Petro	Petroleum Price (PPI)	30	61.92	23.33	31.47	116.50

A. All prices and price indexes are adjusted for inflation and are in constant 1982-1984 dollars.

Table 2: Parameter e**s**timate**s** for the acreage re**s**pon**s**e model

Parameter	Variable	Estimate	Std Err	t Value
λ	Partial adjustment coefficient	0.42	0.16	2.66
α	Intercept	2,843,321	645,278	4.41
β	Rice price ($/cwt.)	173,643	71,069	2.44
γ_1	Soybean price ($/bu.)	-108,915	38,729	-2.81
γ_2	Fuel price (PPI)	-126	2,499	-0.05
γ_3	Fertilizer price (PPI)	-5,107	6,703	-0.76

Table 3: Ela**s**ticitie**s** of rice acreage

Variable	Short-run elasticity	Long-run elasticity
Rice price	0.69	1.19
Soybean price	-0.33	-0.57
Fuel price	-0.006	-0.01
Fertilizer price	-0.19	-0.33

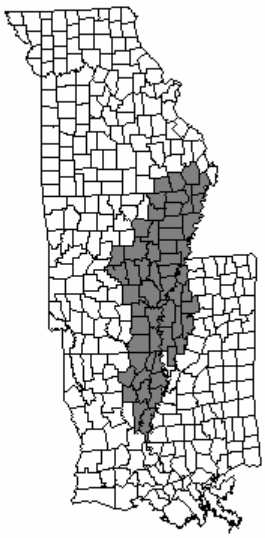

Fig. 1. Counties and parishes included in the Study Region

Investigating the effectiveness of malic acid, nisin, and grape seed extract incorporated into whey-protein coatings to inhibit the growth of *Listeria monocytogenes* on ready-to-eat poultry

Amanda Bettasso[*], *Navam Hettiarachchy*[†], *Vidya Chitturi*[§], *and Michael Johnson*[‡]

ABSTRACT

The ability to control growth of *Listeria monocytogenes* on ready-to-eat poultry products with the antimicrobials nisin, malic acid, and grape seed extract incorporated into whey-protein coatings was evaluated. The antimicrobials were incorporated into the coating solution alone and in combinations. One gram pieces of turkey frankfurters were coated with the coating solutions and then inoculated with *L. monocytogenes* and stored at 4°C for 28 days. The inhibitory effect of the coatings on turkey frankfurter pieces was evaluated on d 0, 7, 14, 21, and 28. Coatings containing 2% malic acid, 3% malic acid, and the combination of nisin (6,000 IU/g) and malic acid (1%) were the most effective in inhibiting the growth of *L. monocytogenes*. Malic acid at 2 and 3% concentrations reduced *L. monocytogenes* population by 2.0 log cycles compared to the control after 28 d. Combination of 1% nisin and 1% malic acid reduced the population of *L. monocytogenes* by 2.7 log cycles compared to the control after 28 d of storage at 4°C. Grape seed extract did not inhibit the pathogen effectively when used alone or in combination with malic acid or nisin. Results of this investigation demonstrate synergistic effects of nisin and malic acid, which can be effectively incorporated into whey-protein coatings to control the post-processing contamination of *L. monocytogenes* in ready-to-eat poultry products.

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[§] V. Chitturi, technical advisor, recently completed a doctorate at the University of Arkansas.

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MEET THE STUDENT-AUTHOR

Amanda Bettasso

After I graduated from Joplin High School in Joplin, Mo., in 2004, I began my studies at the University of Arkansas. I am a senior food science major and have recently completed the AFLS honors program. I have been the recipient of various scholarships while attending the UofA, such as: University Scholarship, Food Science Scholarship, and the OFPA scholarship. I have been a resident assistant for University Housing for the past two years. I am an active member of the Food Science Club and am currently serving as the treasurer. I am a member of the Institute of Food Technology and the Research Chefs Association. I began doing research under Dr. Navam Hettiarachchy during my junior year in the area of food safety. I plan to get my master's degree in food science and eventually obtain a job in research and development.

INTRODUCTION

Listeria monocytogenes is a psychrotrophic, Gram-positive, facultative intracellular organism widely distributed in the environment. It affects pregnant women, immunocompromised patients, newborns, and very old people. *L. monocytogenes* specifically causes listeriosis, which can give rise to meningitis, encephalitis, septicaemia, endocarditis and cause spontaneous abortion, premature birth, stillbirth, abscesses, and lymphadenitis (Kiss et al, 2004). *L. monocytogenes* can survive at temperatures ranging from 2–45°C and in low pH and high osmotic stress, resulting in many challenges for its control in the food industry (Gandhi and Chikindas, 2007). On an average there are 2500 reported cases of listeriosis annually, resulting in about 500 deaths according to the Center for Disease Control (CDC, 2005). *L. monocytogenes* contamination is especially a problem in the ready-to-eat (RTE) meat industry, often causing massive recalls. In February 2007 approximately 52,650 pounds of RTE chicken, processed in South Carolina, was recalled (FSIS, 2007a). There was an outbreak in May 2007 in ready-to-eat turkey products from a poultry ranch in California (FSIS, 2007b). These outbreaks, along with many others, have lead to increased regulations in RTE meats and increased interest in improving the current preservation techniques to inhibit the growth of *L. monocytogenes*.

There are several existing hurdle technologies that attempt to inhibit the growth of *L. monocytogenes* in RTE meats. Thermal processing techniques, although effective, pose the problem of decreasing product quality due to an edge-heating effect that is typically associated with microwave heating (Huang and Sites, 2007). Organic acids are often used in conjunction with other antimicrobials to prevent contamination in food products and have proven to be effective in bacterial inhibition (Palumbo and Williams, 1994; Murphy et al., 2006). Antimicrobial peptides, such as nisin, are used as food preservatives to inhibit growth of several foodborne pathogens and spoilage bacteria in various food products (Zuoxing et al., 2006). Recently, increased interest in plant extracts has grown due to their antioxidant and antimicrobial activities. In addition, there is increasing evidence of antimicrobial properties of phenolic constituents of grape seed extract. Grape seed extract is effective in inhibiting *Escherichia coli* O157:H7, *Salmonella* typhimurium, and *Listeria* (Ahn et al., 2004). Dr. Hettiarachchy's research team focuses on developing protein-based antimicrobial edible films and coatings. This research team showed that the combination of nisin with the natural extracts in edible films could help

increase protections against *L. monocytogenes*, specifically at refrigerated storage temperatures (Theivendran et al., 2006). Heat-denatured whey protein produces bland, flexible, water-based edible films with excellent oxygen, aroma and oil-barrier properties (Perez-Gago and Krotcha, 2001).

With outbreaks occurring as recently as June 2007, resulting in recall of 2,768 pounds of contaminated RTE chicken products out of Tennessee, there is still a call for effective pathogen hurdle technologies (FSIS, 2007c). Hence the objectives of this study are to 1) develop whey-protein edible films incorporated with natural antimicrobials such as grape seed extract, nisin, and malic acid and 2) evaluate their effectiveness in inhibiting *Listeria monocytogenes* in turkey frankfurter systems.

MATERIALS AND METHODS

Listeria monocytogenes was obtained from the Food Microbiology Research Laboratory at the University of Arkansas, Food Science Department. Nisin (N) was purchased from Aplin and Barret Ltd., Dorset, United Kingdom. Grape seed extract (GSE) powder was obtained from Mega Natural Inc., Madera, California. Malic acid (MA) was purchased from Baker, Phillipsburg, N.J. Whey protein was purchased from Land O'Lakes Food Ingredients Division, Arden Hills, Minn., and glycerol plasticizer was purchased from the Sigma Chemical Company, St. Louis, Mo. Turkey frankfurters were obtained from Hettiarachchy's lab, previously made by her team at the University of Arkansas.

Determination of nisin activity. During refrigerated storage nisin loses its activity, hence the activity was determined. *Lactobacillus plantarum* NCDO 955, a standard indicator organism, was used to determine the activity of nisin. Activity was considered as a measure of inhibitory effect of nisin against *L. plantarum* on MRS soft agar plates. Frozen culture of *L. plantarum* was activated by inoculating 10 µl of the culture in MRS medium and incubating at 30°C for 24 h. Approximately 6 ml of MRS soft agar (preboiled at 45°C and cooled) were inoculated with 10 µl of *L. plantarum* and mixed gently. Inoculated soft agar was then poured onto MRS agar plates and allowed to solidify for 30 min under laminar flowing conditions. Stock solution of 0.01 g/ml nisin was prepared in sterilized de-ionized water. Serial dilutions from 1:2 to 1:256 were prepared from the stock and 5 µl of serial dilution were transferred into the surface of the agar by a micropipette tip. The spotted plates were dried for 30 min under the laminar hood and then incubated at 30°C for 24 hours. The activity of nisin was determined using the inverse value of the highest dilution (D) that produced at least a 2mm zone of inhibition. Nisin activity was calculated using the formula:

$$\frac{\text{Amount of sample (5 µl) spotted}}{\text{Inverse of the highest dilution (D)}}$$

Whey-protein film preparation. Nine grams of whey protein were added to 91 grams of water and stirred on a magnetic stirrer continuously until the protein dissolved. Then, 45% glycerol (w/w of protein) was added during continuous stirring for 30 min. The solution was heated at 85°C to denature the protein and to cross link glycerol with the denatured protein to form a polymer. Solutions were cooled to ambient temperature. Nisin (N) was added at 6000, 12,000, and 18,000 IU/g; malic acid (MA) or grape seed extract (GSE) was added at 1%, 2%, and 3% concentrations alone. Concentration of these treatments below 1.0% was not effective in inhibiting the pathogen (Theivendran et al., 2006). Concentrations more than 3.0% resulted in formation of gels in the solution, which is not desirable for coating the food products. Hence the concentrations selected in this study reflect optimum levels for film formation. The combinations of nisin (6000 IU/g), MA (1%), and GSE (1%) were incorporated and stirred until the ingredients were completely solublized. The coating solutions were then stored at 4°C until the meat samples were ready to be coated.

Activation of frozen L. monocytogenes for inoculation of meat samples. The strain V7 serotype 1/2a of *L. monocytogenes* was used in this project. A loopful of *L. monocytogenes* from stock frozen to -70°C was placed in 10 mL of BHI broth and incubated at 37°C for 24 h. A subculture was prepared with 10µL from the first culture in 10mL of BHI and incubated at 37°C for 18 h. Then 1000 µL of this culture were centrifuged for 10 minutes at 7000 xg. Serial dilutions of the cell suspensions were made to obtain an inoculums size of 107 (CFU/ml).

Turkey frankfurter preparation. Turkey breast halves were obtained from Cargill Inc. Minneapolis, Minn. Composition of the turkey frankfurters was as follows: Meat (52.35%), fat (21%), salt (1.25%), phosphate (0.4%), and water (25%). All the dry ingredients, mea,t and water were blended under vacuum for 30 min at 45 rpm with a 220-kg Keebler mixer (Keebler Engineering, Inc., Chicago, Ill.) to form a meat emulsion batter. The meat batters were subsequently stuffed into non-permeable casings (4.6 cm in diameter) and cooked in water tanks for 2 h at 85°C until products reached an internal temperature of 74°C. The frankfurters were chilled in a 4°C cooler. After chilling, the frankfurters were stripped of their casings and sliced into 1-gram pieces (1x1x1cm cubes), packed in sterile Whirl-Pak® bags, and stored at -20°C until used.

Preparation of meat samples. To initiate the studies, the turkey frankfurters were thawed, then dipped in the whey-protein coating (WPI) solutions and dried under laminar flow conditions. A control sample without WPI coating and a control sample with WPI coating with no added antimicrobials were also included. A total of 225 samples were prepared (15 treatments X 3 replications X 5 sampling days). The concentrations and treatments are given in Table 1. After the protein-coated meat pieces were completely drip-dried, they were inoculated with *L. monocytogenes* by dipping them into the cell suspensions in phosphate-buffered saline (PBS) (approximately 10^7 CFU/ml) for one min and dried under laminar flow conditions. Then the meat pieces were packed in sterile Whirl-Pak® bags and incubated for 28 d at 4°C.

L. monocytogenes count determinations in turkey frankfurter samples. The samples were analyzed for survivors of *L. monocytogenes* on d 0, 7, 14, 21, and 28. Samples were crushed in a stomacher with 9.0 ml of PBS. Serial dilutions of the stomached samples were spread-plated on *Listeria*-selective agar. The plates were incubated at 37°C for 48 h and colony-forming units were counted. The plate counts were then analyzed to determine if there were log reductions in *L. monocytogenes* growth.

RESULTS AND DISCUSSION

Inhibitory effect of whey-protein films. At zero hour, mean population of *Listeria* on the frankfurter samples was 5.0 log CFU/g. As shown in Figure 1, microbial counts, in the control without whey-protein coating (C) and in the control with whey protein coating without antimicrobials (C1), increased over the 28 d study. The control sample started with a listerial level of approximately 5.0 log CFU/g at zero hour and increased to 7.85 log CFU/g on d 28. Control 1 had the same initial listerial level as C and grew to 8.12 log CFU/g after 28 d.

WPI coatings with nisin (6000 IU/g, N1) on frankfurter pieces did not inhibit the pathogen effectively when compared to the controls. As shown in Figure 1, the listerial count started at the same level as the control (C) and increased to 7.9 log CFU/g after 28 d. WPI coating with nisin (12,000 IU/g, N2) on frankfurter pieces lowered the *Listeria* population to 4.7 log CFU/g on d 14. This was a 3.7 log reduction compared to the control (C). However, after d 14 the *L. monocytogenes* counts steadily increased to 7.0 log CFU/g after storage for 28 d at 4°C. This can be explained as the development of resistance to the nisin by the *L. monocytogenes* population. The WPI coating with nisin (18,000 IU/g, N3) had a similar pattern to N2, but was more effective in inhibiting the pathogen. The population of *L. monocytogenes* in N3 samples increased over the first 7 d to 7.1 log CFU/g, and then decreased over the next 14 d (from d 7 to d 21) to 4.3 log CFU/g. The L. monocytogenes population developed resistance to the nisin at this concentration and increased to 5.8 log CFU/g at 28 d. The N2 (12,000 IU/g) and N3 (18,000 IU/g) treatments lowered the *Listeria* population by 0.8 and 2 log CFU/g, respectively, compared to the control (C) after 28 d of storage.

Figure 2 shows the response of *L. monocytogenes* in turkey frankfurters coated with WPI films containing malic acid at 1.0% (MA1), 2.0% (MA2), and 3.0% (MA3) concentrations. Listerial counts on frankfurter samples with MA1 steadily increased over the 28 d period to 7.7 log CFU/g. Listerial counts on frankfurter samples containing MA2 and MA3 increased to 7.1 log CFU/ml on d 7 and the decreased over the next 21 d to 5.5 and 5.3 log CFU/g in MA2 and MA3, respectively. There was approximately a 2.0 log CFU/g reduction after 28 d in comparison to the control (C) in frankfurter samples coated with WPI containing 2% and 3% malic acid.

In contrast to nisin and malic acid, the samples containing grape seed extract (GSE) did not inhibit the pathogen. All the GSE treatments at 1.0, 2.0, and 3.0% concentrations (GSE1, GSE2, and GSE3, respectively) had similar growth patterns as the control. The population of *L. monocytogenes* on turkey frankfurter samples coated with WPI containing GSE at 1.0, 2.0, and 3.0% concentrations increased to 8.53, 8.58, and 8.61 log CFU/g, respectively, as shown in Figure 3. These results suggest that grape seed extract is not effective in inhibiting *L. monocytogenes* on turkey frankfurters coated with the WPI films. This can be explained as the availability of protein molecules as nutrients and availability of oxygen conditions, which provide a conducive environment for the growth of *L. monocytogenes*. Previous studies by Dr. Hettiarachchy's team have demonstrated inhibitory effects of grape seed extract incorporated edible films in pre-processing contamination conditions (inoculation of the pathogen on meat samples and then coating the samples with antimicrobial edible films) (Theivendran et al 2006; Chitturi, 2008). However, grape seed extract incorporated WPI films are not effective in controlling post-processing contamination of *L. monocytogenes* on turkey frankfurters.

Figure 4 shows growth of *L. monocytogenes* in turkey frankfurters coated with WPI films containing the combination of N, MA, and GSE. Treatments GSE+MA and N+GSE had similar listerial counts as the controls, C and C1. Both these treatments had listerial counts starting at 5.0 log CFU/g at zero hour and grew to 8.0 and 8.1 log

CFU/g in samples containing GSE+MA and N+GSE, respectively, after 28 d of storage. Listerial levels in the samples containing N+MA increased from 5.0 log CFU/g at zero hour to 6.3 log CFU/g on d 21. After d 21, the listerial level decreased to approximately 5.2 log CFU/g. There was a 2.7 log CFU/g reduction compared to the controls after 28 days of storage. The WPI coatings containing a combination of N and MA were more effective in inhibiting the pathogen than when either was used alone. This can be explained due to the improved stability of nisin at acidic pH. Similar observations were made by Ko et al. (2001), who demonstrated that nisin-incorporated edible films have more potency at lower pH conditions. Moreover, pore formation by nisin on the membrane of *L. monocytogenes* facilitates the lower molecular-weight compound malic acid in penetrating inside the cell and lowering the pH of the cell. This leads to a reduction in listerial levels.

The combination of all three antimicrobials, N+MA+GSE, resulted in a decrease in listerial levels over the first 7 d from 5.0 log CFU/g to 3.7 log CFU/g. The listerial levels then steadily increased over the 28 d storage to 6.8 log CFU/g. There was a 1.0 log CFU/g reduction in the *Listeria* population in comparison to the control after 28 d of storage. Incorporation of grape seed extract with N and MA did not further enhance antimicrobial activities. This could be explained due to the lack of inhibition from the grape seed extract.

In summary, nisin (12000 IU and 18000 IU) and malic acid (2.0 and 3.0%) reduced the *L. monocytogenes* population by 2.0 log CFU/g in turkey frankfurters after 28 d of storage as compared to the controls. Grape seed extract did not inhibit the pathogen at all when used alone or in combination with nisin and malic acid and compared to the control. The most effective treatment in the study was the nisin and malic acid combination (N+MA), which lowered the population of *L. monocytogenes* by 2.7 log CFU/g, compared to the control, after 28 d of storage. Edible films can be an effective hurdle technology against foodborne pathogens and could be used in the ready-to-eat poultry and meat industry.

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Table 1. Composition and concentrations of treatments used in whey protein edible films

#	Treatment	Abbreviation	Concentration (%)		
			N	MA	GSE
1	Control with no WPI film, no antimicrobial additives	C	-	-	-
2	Control with WPI film, no antimicrobial additives	C1	-	-	-
3	WPI film + nisin	N1	6,000 IU/g	-	-
4	WPI film + nisin	N2	12,000 IU/g	-	-
5	WPI film + nisin	N3	18,000 IU/g	-	-
6	WPI film + grape seed extract	GSE1	-	-	1.0
7	WPI film + grape seed extract	GSE2	-	-	2.0
8	WPI film + grape seed extract	GSE3	-	-	3.0
9	WPI + malic acid	MA1	-	1.0	-
10	WPI + malic acid	MA2	-	2.0	-
11	WPI + malic acid	MA3	-	3.0	-
12	WPI + Malic acid + grape seed extract	MA+ GSE	-	1.0	1.0
13	WPI + nisin + malic acid	N + MA	6000 IU/g	1.0	-
14	WPI + nisin + grape seed extract	N + GSE	6000 IU/g	-	1.0
15	WPI + nisin + grape seed extract + malic acid	N + GSE + MA	6000 IU/g	1.0	1.0

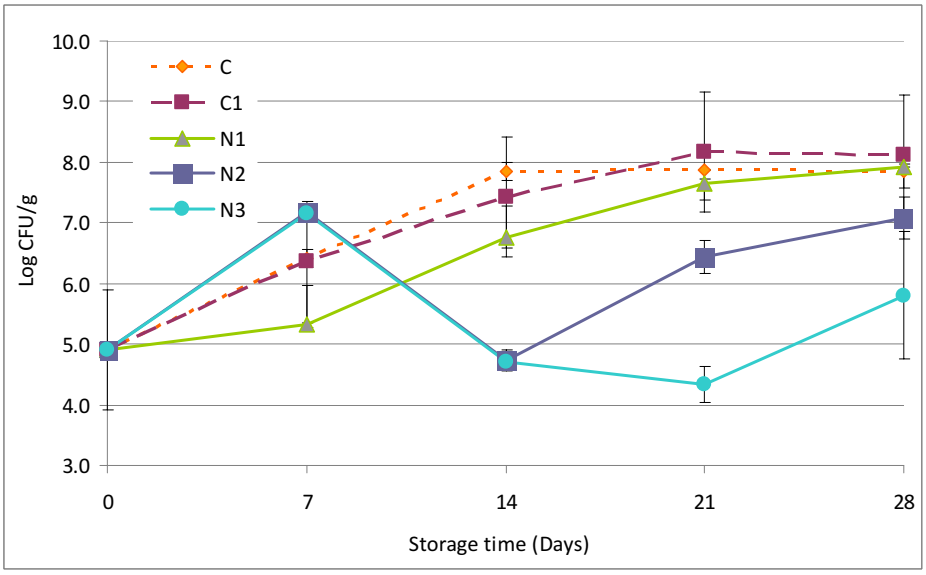

Fig. 1. Inhibitory activity of N-incorporated whey-protein coatings on turkey frankfurters, inoculated with *L. monocytogenes* stored at 4 °C for 28 d. C: Control without WPI films, C1: control with WPI film and without antimicrobials, N: WPI film with nisin at 6,000 IU/g = N1, 12,000 IU/g = N2, and 18,000 IU/g = N3. Values in figure are means of three different replications, error bars represent standard error.

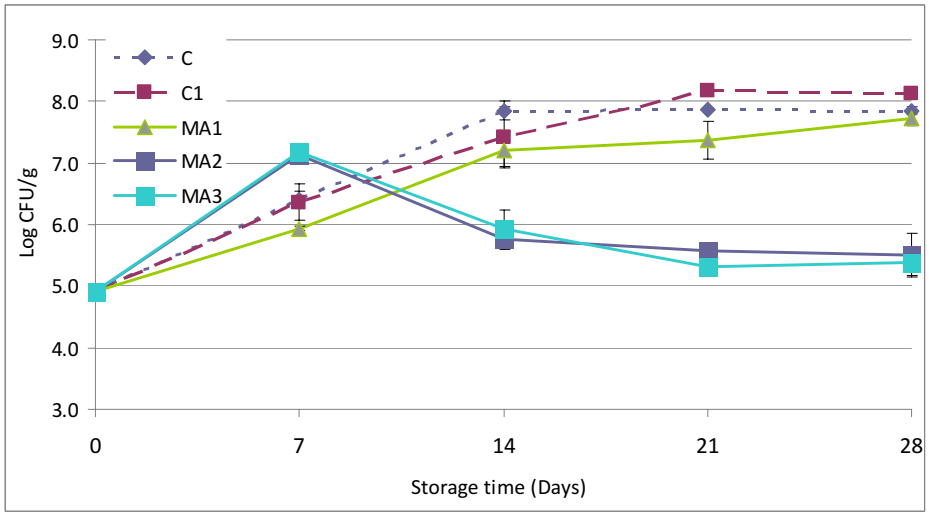

Fig. 2. Inhibitory activity of MA-incorporated whey protein coatings on turkey frankfurters, inoculated with *L. monocytogenes* stored at 4 °C for 28 d. C: Control without WPI films, C1: control with WPI film and without antimicrobials, MA: WPI film with malic acid at 1% = MA1, 2% = MA2, and 3% MA3. Values in figure are means of three different replications, error bars represent standard error.

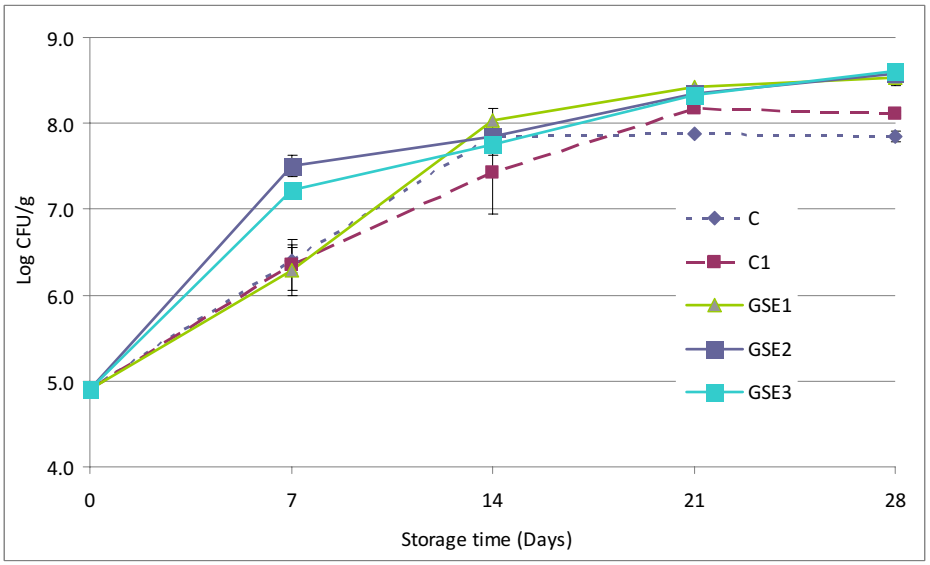

Fig. 3. Influence of GSE **incorporated into whey protein coatings on turkey frankfurters, inoculated with** *L. monocytogenes* **stored at** 4 **°C for** 28 **days. C: Control without** WPI **films, C1: control with** WPI **film and with out antimicrobials**, GSE: WPI **film with grape seed extract at 1**% = GSE**1**, 2% GSE2, **and** 3% GSE3**.** Values in figure are means of three different replications, error bars represent standard error

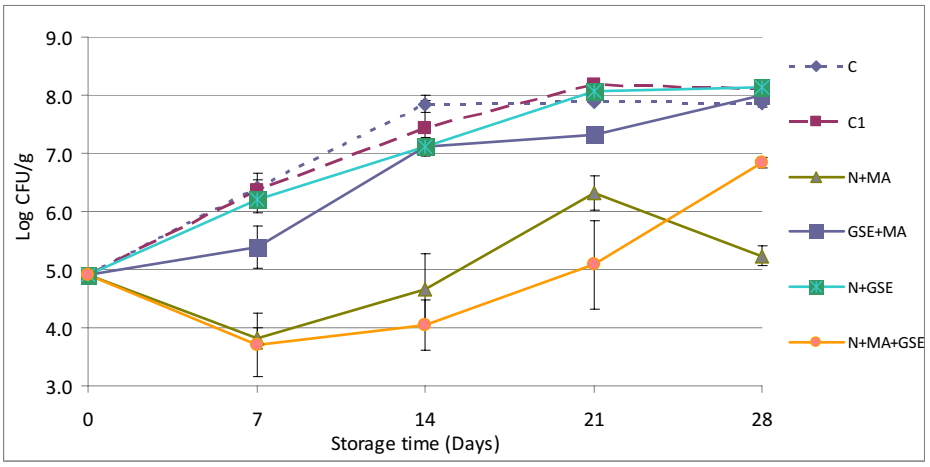

Fig. 4. Inhibitory activity of combinations of GSE,N,and MA into whey protein coatings on turkey frankfurters, inoculated with *L. monocytogenes* stored at 4 °C for 28 d. C: Control without WPI films, C1: control with WPI film and without antimicrobials. C: Control without WPI films, C1: control with WPI film and with out antimicrobials, GSE: grape seed extract, MA: malic acid, N: nisin Values in figure are means of three different replications, error bars represent standard error.

Psycho-social effects of a brain-training program among healthy older adults

Desma J. Hurley[*]*, M. Jean Turner*[†]*, and William C. Bailey*[§]

ABSTRACT

Grounded in cognitive neuroscience and social exchange theory, this research evaluated the relationship between changes in cognitive functioning and two psycho-social dimensions of life among healthy adults over the age of 70 (N=12). Specific psycho-social dimensions examined were social interaction and depression. Six females and six males participated in the study. All were white, college-educated individuals residing in a life-care residential retirement community. The participants used the Posit Science® Brain Fitness Program™, an auditory-based computer training program that improves memory and speed of processing, for forty hours over an eight-week period. Pre- and post-tests related to social interaction and depressive symptoms indicated that improvement in cognitive functioning was related to improvement in psychosocial dimensions in later life.

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MEET THE STUDENT-AUTHOR

Desma Hurley

After graduating from Flippin High School in Marion County, Ark., I enrolled at Arkansas State University of Mountain Home, Ark. From this institution, I earned an associate degree in business with an emphasis in computer applications. After graduating, I became Montessori-certified and a lead teacher at the Fayetteville Montessori School, supervising 18 two-year-olds and six teacher assistants. After working with children for several years, I made the decision to earn my bachelor's degree in Human Development, Family Sciences and Rural Sociology with a Lifespan / Family Sciences concentration. Late in my undergraduate work I became fascinated with research focused on improving the overall quality of life of older individuals. This interest afforded me the opportunity to study cognitive functioning as it correlates with psychosocial effects of mature adults. I am now a first-year master's student majoring in Human Development, Family Sciences, and Rural Sociology with an emphasis in Gerontology. I am currently a graduate research assistant working with M. Jean Turner, Ph.D. I would like to give a very special thank you to my research mentor, Dr. Turner, for her support, motivation, and guidance. Her constant positive support has led me to continue my pursuit of academic achievement.

INTRODUCTION

This study examines how changes in age-related cognitive functioning associated with a brain training program affect depressive symptoms and social interaction among older adults. The purpose of the research is to assess the effects of a brain training program on improved psychosocial functioning of healthy aging adults. This study examines how healthy, mature adults' improvements in cognitive functioning, resulting from use of a computer program, relate to psycho-social dimensions of older adults' lives.

Aging adults often experience "age-related cognitive decline" (ARCD) (Park, O'Connell, and Thomson, 2003). According to researchers, an aging individual experiencing ARCD is also susceptible to decline in his or her psycho-social functioning (Biringer et al., 2005; Cumijs, Jonker, Beekman, and Deeg, 2001; Park et al., 2003). These declines can impact an older adult's level of depression, social interactions, and overall well-being. Research has also shown that ARCD decreases one's short-term memory skills, impacting mental and physical health (Park et al., 2003). ARCD can be a contributing factor to increased levels of depression leading to less social activity for older adults (Biringer et al., 2005; Cumijs et al., 2001).

A computer-based software program called the Brain Fitness Program™ (BFP) by Posit Science® Corporation (San Francisco, California) is designed to stop or even reverse ARCD. This program is a computer-based auditory training software program. The BFP was designed by leading scientists to provide the brain with six specific exercises to improve cognitive ability. The goal of the targeted brain exercises is to drive learning, focus on certain areas of ARCD, and stimulate analytical pathways for an overall increase in cognitive ability. Past research has focused only on cognitive gains related to the BFP's training designs and its core applications for older adults (Mahncke, et al., 2006). The current study explores implications of BFP on the number of depressive symptoms and social interaction.

Social exchange theory provides a possible explanation for the decline in social interaction and activity sometimes seen in elders in later life. This theory sug-

gests that decline of social interaction does not result from one's personal choice or social system needs, but rather from an unequal communication process between older adults and other members of society (Hooyman and Kiyak, 2008). The equal balance of social exchange experienced between an older adult and other individuals establishes the older adult's level of personal satisfaction. Because of the changes in social arrangements, roles, and skills that usually accompany aging, an older individual may have fewer commonalities with his or her social environment. This circumstance may lead to a decline in social interaction experiences. With fewer opportunities to exert power in social interactions, mature adults may remove themselves from interactions and thus be forced to accept a decline in their level of social interaction (Hooyman and Kiyak, 2008).

Older adults who feel a sense of declining cognitive functioning often become less confident during social situations (Plehn, Marcopulos, and McLain, 2003). Those who suffer from ARCD often experience less personal independence and a decline in overall quality of life (Plehn et al., 2003). The BFP used in this research may provide gains in cognitive functioning that lead to improvements in social interaction as well as decreases in the number of depressive symptoms participants experience.

Cognitive Functioning. ARCD is the normal progression of memory and cognitive decline among older adults, affecting more than 36% of adults 85 years of age and older (Black and Rush, 2002). The greatest amount of cognitive decline occurs among the oldest-old cohort, the fastest growing population in the United States (Park, et al., 2003). A study of 15,000 participants conducted by Cutler and Grams (1988) found that 40% of individuals over 55 years of age experienced a decline in memory skills.

An individual experiencing ARCD requires more time to learn information than his or her counterpart. While conversing, one may have difficulty conjugating words and may often repeat sentences because he or she can recall thinking a statement but not recall expressing it (Insel and Badger, 2002). Mature adults who suffer from ARCD often experience a loss of independence and a decrease in overall quality of life (Plehn, et al., 2003). Cognitive decline is a predictor of functional disability (Doge, Du, Sazton, and Ganguli, 2006). Also, those who suffer from ARCD are more likely to be institutionalized and require more medical services than other individuals (Black and Rush, 2002). Doge et al., (2006) expressed that cognitive decline is coupled with a decrease in instrumental activities of daily life (IADLs). IADLs include money management, cooking, laundry, and simple household cleaning (Plehn, et al., 2003).

Depression. Research suggests that ARCD leads to a decline in mental health and social interaction (Mahncke, et al., 2006). Depression and ARCD are directly correlated with one another. Hofman, et al. (2000) found an inverse association between levels of depression and cognitive functioning. Intervention programs that increase cognitive functioning may also prevent the development of mental illnesses like depression among older adults (Blazer, 2002).

Depressive symptoms include: feelings of sadness and loneliness, sleep disturbance, lack of interest, energy reduction, changes in appetite, and concentration difficulties (Blazer, 2002; Insel and Badger, 2002). Older adults with symptoms of depression often experience a drop in cognitive functioning, a decrease in information processing speed, and declines in memory, abstract reasoning, flexible thinking, and word creation (Biringer, et al., 2005; Blazer, 2002). Depression can be compounded with the onset of ARCD because of a decline in overall self-efficacy (Biringer, et al., 2005).

Social Interaction. ARCD has been reported to be negatively related to social interaction (Nezlek, Richardson, Green, and Schatten-Jones, 2002). A persistent and healthy social life is more beneficial to older adults than it is to any other age group (Caprara and Steca, 2005). It greatly increases the overall life satisfaction of elders (Caprara and Steca, 2005). Adults in late life who report a rewarding, active, high-quality social life also report greater psychological well-being than other individuals. In return, healthy psychological well-being is positively related to the quantity and quality of social interaction (Nezlek, et al., 2002). Engaging in social activity is likely related to better physical and mental health, and may also be a predictor of fewer disabilities and a decrease in overall ARCD (Doge et al., 2006).

A common denominator of social interaction is loneliness. Older adults spend more time alone than individuals of other age groups (Adams, Sanders, and Auth, 2004). Loneliness is defined as a reaction to a difference between desired and achieved social activity (Blazer, 2002). Some characteristics of loneliness include shyness, low self-esteem, self-depreciation, and poor social skills, some or all of which may stem from childhood (Blazer, 2002). A decline in social activity is associated with depression. Further, depressive symptoms may lead to lower levels of overall social activity. Loneliness is not a symptom of depression. However, it may contribute to depression (Adams et al., 2004). Mature adults can relieve or prevent loneliness by enhancing their cognitive functioning (Blazer, 2002).

Brain Fitness Program. The BFP was designed to strengthen brain health and quality of life for older individuals. As people improve their abilities to hear and

process language, they also improve their abilities to remember information and act on what they hear. The BFP guides users away from learned negative behaviors that diminish brain health into new behaviors that positively reinforce improved brain-functioning skills. Each BFP exercise targets specific aspects of cognitive decline, aiming to reduce or even reverse ARCD. Participants who have completed the BFP report improved communication skills, memory, self-confidence, attention, and optimism (Posit Science, 2006).

Components of the Brain Fitness Program. The BFP is composed of six basic exercises that build cognitively on each other. For example, the first exercise, High or Low, is a time-order judgment exercise. The goal of this exercise is to gradually improve one's ability to identify sweeps and bursts that are shorter and close together in time, training the brain to react faster and more accurately to sounds. The fourth exercise, Sound Replay, is a serial memory-span exercise. This exercise is designed to improve the brain's ability to differentiate sounds, store them, and recall increasingly longer series of sounds or syllables in their correct sequence (Posit Science, 2006).

The BFP has been shown to decrease and sometimes reverse the effects of ARCD. As stated previously, ARCD can negatively affect the overall quality of life and sense of well-being for older adults. Recovering from ARCD may lead to an increase in social interactions, mental health, and self-efficacy, and may reduce depressive symptoms. Mahnke, et al. (2006) found the BFP to be effective with adults over 60 years of age. Test results for those scoring below 85% on the initial neurological assessments indicated that the test group experienced increases in the areas measured, showing that the brain plasticity-based program improved cognitive functioning (Mahncke, et al., 2006).

MATERIALS AND METHODS

The objective of this research project was to determine psycho-social changes associated with the use of the BFP among healthy, community-dwelling, mature adults. The relationship between changes in cognitive functioning, depressive symptoms, and sociability is examined using pre- and post-test measures. The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS; Randolph, 1998) was used for cognitive evaluation. Dr. Stephen Gemmell, a neuropsychologist, and Ms. Nakia Richter, a testing and measurement technician, conducted the pre- and post-test assessments using the RBANDS to assure consistency and accuracy in cognitive evaluations for the study. Social interaction and depressive symptoms were assessed using the Form 36 HARQoL (Brazier et al., 1992). Participants were also asked about the frequency and extent to which heath problems interfered with social interaction.

Older adults (N=12) living in a multi-level retirement community were recruited and trained on the BFP. All potential participants were determined to be free of diagnosable cognitive decline and depression. Participants were instructed to use the BFP once a day for one hour, five days a week, for eight weeks for a total of 40 hours. The student researcher loaded the program onto the study group's personal computers and demonstrated how to use the software. The researcher then remained in contact with the participants as frequently and in the manner each individual chose in order to resolve any problems with the BFP. Some participants requested regular weekly visits, others wanted phone calls on a regular basis, and still others requested e-mail contact as they needed it.

RESULTS AND DISCUSSION

A one-tailed t-test was used to assess positive change between time one and time two. Due to the small sample size and the fact that this project is exploratory research, the acceptable level of significance was set at $p < .10$. Although changes in the participants' levels of cognitive functioning were not statistically significant, the post-test results indicated improvement in several cognitive dimensions measured (see Table 1), as predicted by previous research (Mahncke, et al., 2006). Results also indicated a significant reduction in the participants' perceptions of the extent to which health problems interfered with social activities between time one and time two (see Table 2). However, no significant reduction in their perception of the frequency with which health problems interfered with social activities was found. Only one indicator of depressive symptoms approached significance. Respondents reported fewer instances of feeling down in the dumps following their experience with the BFP (see Table 3).

This study provides important insight into a previously unexplored area of research. However, it does have some limitations. The biggest limitation is the sample size. Because it serves as an exploratory study, the sample was limited to a manageable and conveniently available group. The sample was primarily obtained from a local multi-level living facility by asking for volunteers. The self-selection process combined with the high level of education and SES of the residents and numerous available activities provided by the center make it difficult to generalize the findings of this study to a broader population of older adults. To further explore the issues of the study, future research needs larger, more diverse

samples reflecting both community dwelling and residential elders.

The findings of this study provide a beginning for understanding the relationship between improving cognitive functioning and social interaction and the number of depressive symptoms experienced by older adults. Future research is required if we are to fully understand associations between improved brain fitness and overall well-being for aging individuals.

Individual reports from participants indicated that they perceived more of an increase in cognitive functioning and social interaction as well as a greater decrease in depressive symptoms than the statistical analysis indicated. Anecdotal comments of participants include "I do not have to write down telephone numbers from the phonebook anymore. I can remember the numbers as I walk from the phonebook to the telephone." "Installing the BFP onto my computer early this summer allowed me time to gain the needed confidence to participate in the play at the end of the summer. Both my wife and I are impressed that I remembered 40 lines." Participating in the BFP gave this 92 year-old individual the confidence to pursue being in a play, which he very much wanted to do. However, before participating in this study he had doubted his ability to memorize lines should he join the cast.

This project examines a modern-day application for enhancing older adults' cognitive abilities. The positive effects on cognitive functioning are well documented in previous research (Mahncke, et al., 2006). However, previously only anecdotal evidence supported the expectations of this study—that cognitive improvements would also improve various psycho-social dimensions. Increasing social interaction and reducing the number of depressive symptoms experienced combine for an overall effect of enhancing quality of life among older adults. Combining new technology with the potential to enrich overall well-being for the rapidly growing older adult population will create greater health and fitness opportunities for many future generations. Discovering methods to maintain cognitive ability leading to the reduction of depressive symptoms and loneliness while increasing social interactions and adaptability will improve quality of life for mature adults as well as for the loved ones who care for them.

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Table 1. Cognitive Functioning

BFP Factors	Pre-test mean(SD)	Post-test mean(SD)	t	p-value
List recall	4.83(1.56)	5.25(.329)	-0.959	0.179
Delayed memory	98.25(17.062)	102.33(11.139)	-0.867	0.203
Figure recall	11.33(2.807)	10.92(3.872)	0.302	0.384
Semantic fluency	17.75(2.989)	18.83(3.010)	-1.193	0.129

Table 2. Social Interaction

Perceived Health Interference	Pre-test mean(SD)	Post-test mean(SD)	t	p-value
Extent health problems interfered with social activities	4.58(.669)	4.00(1.044)	2.028	0.034*
Frequency health problems interfered with social activities	4.18(1.25)	4.55(.688)	-0.938	0.186

*Significance level p <.10.

Table 3. Depression

Depressive Symptoms	Pre-test mean(SD)	Post-test mean(SD)	t	p-value
Been very nervous	4.36(.674)	4.27(.647)	0.363	0.362
Felt so down in the dumps that nothing could cheer you up	4.64(.674)	4.82(.182)	-1.491	0.084*
Felt calm and peaceful	3.82(.982)	3.73(1.009)	0.209	0.420
Felt downhearted and depressed	4.45(.522)	4.55(.522)	-0.559	0.294
Been happy	4.00(.447)	3.91(1.044)	0.219	0.416

*Significant level p< .10.

Using combined prediction models to quantify and visualize stormwater runoff in an urban watershed

Keshia M. Koehn[*]*, Cristina M. Scarlat*[†]*, and Kristofor R. Brye*[§]

ABSTRACT

Stormwater runoff can transport nutrients, sediments, chemicals, and pathogens to surface waterbodies. Managing runoff is crucial to preserving water quality in rapidly developing urban watersheds like those in Northwest Arkansas. A watershed containing the majority of the University of Arkansas campus was designated as the study area because stormwater from it drains into the West Fork of the White River, designated as an impaired waterbody due to siltation. The project objective was to develop methodology to test existing stormwater drainage infrastructure, identify potential areas of improvement, and estimate potentially contaminated runoff by combining two widely used prediction models. The U.S. Department of Agriculture's Natural Resource Conservation Service's curve number (CN) method was used to estimate runoff depths and volumes, while a flow-direction model was created that integrated topography, land use, and stormwater drainage infrastructure in a geographic information system. This study combined the CN and flow-direction models in a single geodatabase to develop flow direction/quantity models. Models were developed for 5-, 10-, 25-, 50-, and 100-year floods and varied by the antecedent moisture content. These models predicted flow directions within existing drainage infrastructure and runoff volumes for each flood, and served as a hypothetical flood analysis model. Results showed that between 24,000 m^3 (5-year flood) and 60,000 m^3 (100-year flood) of runoff would be transported to the West Fork of the White River. The methodology developed and results generated will help stormwater planners visualize localized runoff, and potentially adapt existing drainage networks to accommodate runoff, prevent flooding and erosion, and improve the quality of runoff entering nearby surface waterbodies.

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INTRODUCTION

After a precipitation event, stormwater runoff transports nutrients, chemicals, sediments, and pathogens to surface waterbodies. Managing this stormwater is crucial in preserving water quality, especially in urban watersheds under heavy development like in Northwest Arkansas, specifically Benton and Washington counties. Northwest Arkansas is the home of several large-scale corporations such as Wal-Mart, Tyson Foods, and J.B. Hunt Transportation. According to the 2000 Arkansas Census, Benton County's population experienced a 57% increase from the 1990 Arkansas Census and Washington County has experienced a 39% increase, an increase totaling over 300,000 people in both counties combined (US Census, 2000).

Washington County is also home to the University of Arkansas, the state's land-grant institution. Since 2003, more than nine buildings have been restored and more than twenty buildings have been erected at the University of Arkansas (FMPG, 2007). Of these newly constructed buildings, three dormitories were built to house the increasing student population. Like the population growth in Northwest Arkansas, student enrollment at the University of Arkansas has increased by 21%, from 15,396 in 2000 to 18,647 in 2007 (Voorhies, 2007).

The majority of stormwater runoff within the subwatershed surrounding the University of Arkansas in Fayetteville flows into the West Fork of the White River; both the White River and its West Fork are tributaries of Beaver Lake, the source for much of Northwest Arkansas' municipal water supplies. However, the West Fork of the White River has been identified as an impaired stream by the Arkansas Department of Environmental Quality due to "high turbidity levels and

MEET THE STUDENT-AUTHOR

I graduated in 2005 from the Arkansas School for Mathematics, Sciences, and the Arts in Hot Springs. I am a junior double-majoring in environmental, soil, and water science in the Dale Bumpers College of Agricultural, Food and Life Sciences and in geology in the J. William Fulbright College of Arts and Sciences. I was able to attend the University of Arkansas after receiving the Honors College Academy Scholarship in addition to several private scholarships provided by the Dale Bumpers College and the Department of Crop, Soil, and Environmental Sciences.

Keshia Koehn

I have worked two different jobs since I was a freshman, including at the Center for Advanced Spatial Technologies (CAST) at the University of Arkansas as a GIS technician. I have also been a member of the Crop, Soil, and Environmental Sciences undergraduate student club and the National Society for Collegiate Scholars since my freshman year. After graduation, I plan on applying to a doctoral program in hydrogeology to work on groundwater system contamination and analysis.

This research was completed as part of the requirement for my undergraduate honors thesis. This project was funded by the State Undergraduate Research Fellowship (SURF) and a Dale Bumpers Research Grant. Initially, the University of Arkansas Facilities Management contracted CAST to update the existing stormwater drainage network. The task developed into the project of analyzing stormwater runoff for the majority of the campus area. I have presented this research at five conferences and won 2nd place at the Southern Branch American Society of Agronomy (ASA) Graduate Oral Symposium and 1st place at the Gamma Sigma Delta Undergraduate Oral Symposium.

excessive silt loads" that were creating an aquatic environment incapable of supporting adequate life (ADPC&E, 1998).

After the West Fork of the White River was placed on the Arkansas 303(d) list for impaired waterbodies in 1998, it was determined that sediment loads were originating from a variety of sources including stream bank erosion, local construction and development, pastures, forests, and urban areas (Formica et al., 2004). According to a best management practice (BMP) study by the US Environmental Protection Agency (EPA), average sediment contributions to surface waterbodies ranged from 213 million mg ha^{-1} yr^{-1} (190 lbs ac^{-1} yr^{-1}) in medium-density residential areas to 1.21 billion mg ha^{-1} yr^{-1} (1000 lbs ac^{-1} yr^{-1}) in commercial areas (USEPA, 1999). With the current high rate of expansion and development in Northwest Arkansas, strategic modeling and planning of stormwater runoff plays a critical role in preserving the quality of surface water.

One of the most common stormwater runoff prediction models is the U.S. Department of Agriculture's Natural Resource Conservation Service's (NRCS) curve number (CN) method (Thompson et al., 2003). Formerly known as the Soil Conservation Service (SCS) Method, the CN method calculates a net runoff depth for a specific amount of precipitation. This method is based on estimations of net runoff after initial losses of accumulated rainfall due to soil storage, interception, and infiltrated runoff (SCS, 1972).

There are several parameters that are used to determine a CN for an area. The land use of an area, or amount/type of surficial cover, can be used to determine the amount of runoff that can be intercepted and/or infiltrated. For example, a paved area would have greater runoff than a grassy area, which would have greater infiltration. Land use can also include land treatment in agricultural settings where crop rotations, contouring/terracing, and the amount of grazing and burning affect quantity of stormwater runoff (Anonymous, 2007).

Soil properties can also have an effect on depth of stormwater runoff. The hydrologic soils group (HSG) is a classification of soil moisture based on the quantity of water that is able to infiltrate the soil, which is influenced by the condition of the soil surface and the soil profile horizonation and includes slope, texture, and hydraulic conductivity (USDA, 2007). Table 1 provides the HSG classification definitions by the NRCS.

The antecedent moisture content (AMC) is another soil property that has a significant effect on quantity of stormwater runoff. AMC is defined as the level of soil moisture before a precipitation event and is divided into three classes: AMC I, AMC II, and AMC III (Anonymous, 2007). A soil with AMC I conditions is described as considerably dry, but not to the wilting point for plants. A soil having AMC II conditions is described as having an average soil moisture condition, and AMC III conditions correspond to a soil that is nearly saturated (Novotny, 1995).

The amount of precipitation, land use, HSG, and AMC are used in the CN method to calculate runoff for an area. This method operates on the assumption that each soil-land-cover combination produces a separate curve number that can be used on catchment areas up to 1000 km^2 (Williams and LaSeur 1976).

This project was designed to develop a stormwater-runoff prediction model in an effort to simulate non-point source contamination of local rivers, like the West Fork. This study sought to develop a methodology to test existing stormwater-drainage infrastructure and identify potential areas of improvement and to estimate potentially contaminated runoff volumes by combining two widely used prediction models [i.e., the NRCS CN method integrated with a geographic information system (GIS) modeling approach].

With the methodology in place, the objective of this study was to evaluate the effects of AMC on stormwater runoff for 5-, 10-, 25-, 50-, and 100-year flood events. The study area was defined as the portion of the University of Arkansas main campus in Fayetteville that contributes stormwater runoff and potential pollutants to the West Fork of the White River. It was hypothesized that the effects of AMC would increase as the flood-return period increased.

MATERIALS AND METHODS

Study area. The study area was located in the City of Fayetteville, Washington County, Northwest Arkansas, and is a delineated sub-watershed of the NRCS 12-digit Hydrologic Unit Code (HUC) Town Branch – West Fork – White River Watershed (110100010404) (Fig. 1). This sub-watershed has an area of 320 ha (~800 acres) and contains the majority of the University of Arkansas' main campus.

This site was chosen because of the availability of necessary data, the diversity of land uses within the sub-watershed, and because this area is a reasonably representative model of small-scale, rapid development. The region encompassing the actual study area is situated in the Ozark Highlands, where geologic ages of the underlying stratigraphic layers range from Late-Mississippian to Middle-Pennsylvanian sandstone with underlying sequences of shale, siltstone, and limestone. (USGS, 2007). Soil data for the study area, obtained from the NRCS Soil Survey Geographic (SSURGO) Database

(Soil Survey Staff, 2006), indicate the most common soil-surface textural class present in the study area is fine sandy loam. In this area, stormwater runoff can carry sediments from disturbed topsoil in construction zones, chemicals from paints and fertilizers used on athletic fields, pathogens from animal litter in parks and residential areas, and trash, oils, and heavy metals from parking lot runoff.

Data development. A digital elevation model (DEM) extracted from light detecting and ranging (LIDAR) data [7.62 meter (25 ft) resolution] was used for calculations in the flow-direction model. This DEM was made available by the Center for Advanced Spatial Technologies (CAST) at the University of Arkansas and the Northwest Arkansas Regional Planning Commission (NWARPC). Aerial photography obtained in January 2007 [0.15 m (6 in.) resolution] was provided by NWARPC. Soils data for the study area were obtained from the NRCS 2007 SSURGO Database (Soil Survey Staff, 2006). Finally, a five-category impervious surface map was created from the aforementioned aerial photography. The impervious surface map was divided into five land-use categories: impervious surfaces, woodlands, grasses, bare soil, and water.

Positions of stormwater features and infrastructure for the University campus features and City of Fayetteville were identified and differentially corrected (post-processed code) using a Trimble GeoExplorer XT GPS unit (Trimble, Sunnyvale, Calif.). Collected stormwater features included intakes like storm grates, linear grates, culverts, area drains, floor drains, roof drains, and curb inlets. Outflow features included outflow pipes and culverts. Other stormwater features that did not play an active role in the flow-direction analysis of runoff included manholes and cleanout features. Locations and attributes of pipelines were provided by the University of Arkansas Facilities Management and the City of Fayetteville GIS Laboratory.

The study area was delineated from three, NRCS 12-digit HUC watershed boundaries using the LIDAR DEM. The three 12-digit HUC watershed boundaries were selected based on their spatial proximity to the central campus: the Hamestring Creek watershed (111101030203), the Town Branch – West Fork watershed (110100010404), and the Mud Creek – Clear Creek watershed (111101030202).

Flow-direction analysis. The first stage of synthesizing the model was to establish flow directions of stormwater runoff within the existing drainage infrastructure. A flow-direction model will provide a way for planners to assess areas needing improvement and will aid in tracing potential contamination pathways.

Spatial and physical connectivity between stormwater pipelines and features was established after creating a geometric network using ArcGIS version 9.2 [Environmental Systems Research Institute (ESRI), Redlands, Calif.]. Geometric network development enabled the complex linear edges and point features of the existing stormwater-drainage network to operate as a complete system. Weights were added to the geometric network such as pipe lengths, diameters, and elevations. In addition, the material used to construct the infrastructure was recorded. Using ArcHydro, and extension of ArcGIS, these weights were used to design a set of algorithms that were able to establish flow direction within the desired stormwater-drainage network.

After determining flow directions of stormwater runoff within the drainage network, the Utility Network Analyst toolset of ArcGIS was used to determine sample contamination pathways in addition to lengths of hypothetical contamination pathways.

Runoff depth and volumetric quantification. It was necessary to generate a set of spatially distributed CN for the entire study area in order to estimate the potential amount of stormwater runoff for a specific precipitation event. Curve numbers were generated using the NRCS CN method, an impervious surface map, surface topography data from the DEM, soils information for the area, and local precipitation data.

Volumes of runoff were calculated in 50 sub-watersheds using runoff depths from the CN method. ArcHydro [Environmental Systems Research Institute (ESRI), Redlands, Calif.] was used to delineate sub-watersheds based on the stored flow directions within stormwater pipelines and key outflow storm features. Because the stormwater drainage network was used instead of a surface water network, sub-watersheds were developed based on flow directions within the pipelines. The precipitation amount, initial abstraction (ie., amount of runoff lost to infiltration, interception, and possible evaporation), and potential maximum subsurface storage were inserted as additional sub-watershed attributes. These parameters were used to estimate runoff using the CN method. Precipitation data were derived for Northwest Arkansas for five flooding recurrence intervals, 5 (142 mm), 10 (159 mm), 25 (184 mm), 50 (203 mm), and 100 year (225 mm), provided by the U.S. Weather Bureau (1958). These precipitation data were used in the CN method to provide a base level for net runoff.

Potential maximum flood model analysis. The potential maximum flood model was designed to be a hypothetical scenario, created in order to visualize runoff movement through the watershed. To assess potential maximum flooding depths, it was necessary to generate a flood environment confined by the study area bound-

aries. The initial model assumed no addition of runoff water from surrounding watersheds and no loss of runoff water from the study area to surrounding watersheds. The depth of ponding in this model was used to visualize localized runoff and to determine areas that had volumetrically high runoff.

ArcScene (ESRI) was used to create a three-dimensional, seamless model capable of representing the advance and retreat of runoff water in the study area, assuming it was a confined environment. The depressionless DEM, aerial photography, and the sub-watershed and study-area vector boundaries were imported into ArcScene (NAD State Plane 1987 FIPS 301 Feet, Coordinate System). The base heights for the photography and vector boundaries were set equal to the heights of the DEM in order to achieve a seamless, three-dimensional model.

The base heights for the "flood" layer were not set to that of the DEM, but to the minimum elevation of the study area. This boundary created a moveable, planar layer that was able to simulate the flooding capacity of runoff within the study area. This height of the "water" layer was set to the starting position of "No Flooding." For each corresponding flood interval, this layer's base height increased relative to the maximum height of ponded water. ArcScene was also used to model the maximum depth of ponded water for 5-, 10-, 25-, 50-, and 100-year flood recurrence intervals.

RESULTS AND DISCUSSION

Flow direction tracing. The flow-direction prediction model that was developed for this study is important for planning officials at the University of Arkansas and City of Fayetteville at a small scale. However, even though the study area only covered approximately 320 ha (800 acres), the methodology used to develop this flow-direction prediction model can be repeated for larger scales. Since transportation routes of potentially contaminated runoff can be visualized, the model can be used to trace accidental spills, re-route runoff to treatment facilities, and identify locations near outflow features that may be particularly sensitive to contamination.

Curve number analysis. A composite CN map was generated for each level of AMC (AMC I, II, and III) using the impervious surface and soils maps (Fig. 3). Each land-use/soil-group polygon was assigned a CN to be used to calculate a weighted CN average for each sub-watershed. Weighted CN that were calculated for each of the 50 sub-watersheds were used to determine the impact of land use and soils on the amount of direct runoff. The CN maps generated for each AMC condition illustrate not only the abundances of low-permeable land uses in the study area, but also the effects these land uses have on the volume of runoff water being transported into the West Fork of the White River.

Upon observation of the weighted, sub-watershed CN map with reference to the impervious surface map, areas that have the same land-use category, but different HSG, have different local CN. This is shown in the Western quadrant of the study area within the wooded land-use area. This situation can be compared to a sandy soil and a clayey soil under tree cover, because each soil texture has different infiltration and water-holding capacities that affect the amount of runoff. The HSG of an area has a direct effect on the CN-runoff relationship—sandy soils are capable of being more permeable than clayey soils, thus sandy soils have a greater capacity to filter runoff water. These areas are particularly important in filtering contaminated stormwater runoff and are comparable to the drain field of a septic system. Increasing areas that have the capability of runoff infiltration and decontamination can decrease contaminant loading to nearby surface waterbodies, such as the West Fork of the White River.

The mean weighted CN for the sub-watersheds increased with each increase in AMC level. Three maps were developed depicting the weighted CN for each sub-watershed varied by the level of AMC (Fig. 4). The three maps show an increase in the average weighted CN for each increase in AMC level supported by the areal extents of higher CN. There is a clear relationship between the weighted CN and the AMC level—as the soil water content (i.e., AMC) increases, the weighted CN for a watershed also increases, meaning more runoff will occur because the soil has a decreasing capacity to store more infiltrating water as the water content approaches saturation (i.e., AMC III).

The minimum calculated curve numbers for AMC I, II, and III conditions were 52, 71, and 85, respectively. The maximum calculated curve numbers for AMC I, II, and III conditions were 93, 98, and 99, respectively. Average curve numbers for AMC I, II, and III conditions were 79, 89, and 94, respectively.

Runoff depth and volume analysis. The CN method was used to determine net runoff for each sub-watershed for a specific single-storm event. Net runoff increased for each increase in flooding-recurrence interval. As expected, for each increase in precipitation amount, there was a corresponding increase in runoff because of decreasing soil storage capacity. Modeling depths of runoff from precipitation data is important in visualizing the quantity of stormwater being transported by existing infrastructure, given the soil moisture condition (i.e., AMC I, II, or III) at the time of the event.

Also shown by the weighted sub-watershed CN map, for each increase in AMC level, there was a corresponding increase in net runoff for the same precipitation event. Table 2 summarizes the effects of increasing precipitation on the stormwater runoff in each sub-watershed per AMC level.

Net runoff depths calculated from precipitation data were used to calculate volumes of water associated with a specific precipitation event. Runoff data showed that each increase in precipitation was associated with an increase in the volume of runoff water. In addition, the increasing trend in the volume of stormwater runoff was directly related to depths of runoff and to the soil moisture condition (i.e., AMC level). Calculated stormwater runoff volumes were combined with the flow-direction model to visualize maximum flood water retention within the study area.

Maximum flood water retention. In order to model maximum flooding depths of ponded water within the area of study, a hypothetically closed-"bowl" system was constructed to eliminate runoff volume additions and losses to and from surrounding watersheds. Antecedent moisture condition I provided the least areal extent of flooding with increasing flood area in AMC II and AMC III conditions, respectively (Fig. 5). This is directly related to decreasing soil storage capacity as the AMC increases.

For each increase in AMC level, there was an increase in the areal extent covered by ponded water. For lower-magnitude flood-recurrence intervals (i.e., 5 and 10 year), there was a smaller range of area covered by runoff water. For greater-magnitude storm events (i.e., 25-, 50-, and 100-year floods), there is a greater range of areas covered by ponded water (Table 3).

The average height of rise of flood water in the hypothetical closed-"bowl" study area was 0.95 meters (3.11 feet). This is a relatively shallow depth of water, but its magnitude increases greatly when distributed over a low-relief region of the study area. As previously described, this model was conceived in a hypothetically closed system and was able to represent the height of rise and areal extent covered by ponded runoff water. In reality, there would also be simultaneous additions and losses of runoff water in the study area that likely keep maximum flooding depths lower than those predicted by the model.

Significance of research. This project's objective was to develop a methodology to test existing stormwater-drainage infrastructure and identify potential areas of improvement and to estimate volumes of potentially contaminated runoff by combining two widely used prediction models. Using these developed models as a guide, planners at the University of Arkansas and City of Fayetteville can work to improve the quality of runoff water being transported to the West Fork of the White River. Using the flow-direction model and estimated runoff volumes, stormwater-drainage infrastructure can be improved in sub-watersheds that have been shown to contribute the largest volumes of runoff from the study area and in areas experiencing or predicted to experience localized flooding and soil erosion on land and along stream banks.

Currently, stormwater-drainage infrastructure within the study area releases untreated runoff water directly into College Branch Creek, which is a tributary of the West Fork of the White River. During storm events, nutrients, chemicals, sediments, and pathogens are ultimately carried through the drainage network to the West Fork of the White River. The southern reach of College Branch Creek is presently showing severe erosion, and thus is transporting sediment-laden runoff to the West Fork (Fig. 6). This model can help planners re-route runoff away from College Branch Creek, thus likely reducing sediment loads transported to the West Fork.

Future implications. Surficial soil properties and land use have been shown to have an impact on the amount of runoff directly discharging from an area. Models of runoff quantity and flow direction were not only created to determine the quantity of stormwater runoff and its flow through a watershed, but also to develop a reproducible methodology for visualizing small-scale, urban stormwater runoff.

The runoff flow-direction model of the existing stormwater-drainage network is particularly useful for tracing possible point-source contamination. In the case of accidental spills or leaks, planners can accurately trace the contamination pathway through the pipeline infrastructure. In addition, drainage infrastructure surrounding potentially hazardous areas can be modified to transport contaminated water away from surface waterbodies in the likelihood of a spill or leak. Finally, the runoff flow-direction model can be used to decrease localized flooding by re-routing runoff water away from low-relief areas that have greater potential to accumulate runoff from a precipitation event.

Because of the current rate of expansion at the University of Arkansas, construction sites are prevalent throughout much of central campus and contribute to the sediment loading of the West Fork after precipitation. The amount of sediment in stormwater runoff could be decreased by instituting various BMPs that increase the percentage of permeable land, such as permeable pavement and green roofs and a series of detention ponds or grassy swales to slow the water velocity and allow sediment to drop out of suspension before entering College Branch Creek. By constructing moni-

toring stations along key points in the stormwater-drainage network, areas with high sediment or contaminant loads could be re-routed using the flow-direction model within the existing drainage infrastructure.

Conclusions. In rapidly developing urban watersheds, improperly managed stormwater runoff can degrade surrounding surface waterbodies. In Northwest Arkansas, sediment-laden stormwater runoff is transported to the West Fork of the White River, a surface waterbody impaired by siltation. This project established a repeatable protocol that resulted in a stormwater prediction model that was varied by potential soil moisture conditions in an effort to simulate non-point source contamination of local rivers, such as the West Fork, from urban stormwater drainage networks. The AMC of the soils studied had a direct effect on the amount of stormwater runoff from the study area because the soil had a decreasing capacity to store additional infiltrated water as the soil water content approached saturation.

The methodology developed by this research project can be used to test existing stormwater-drainage infrastructure and identify potential areas of improvement and to estimate the volume of potentially contaminated runoff. The runoff flow-direction model will be particularly useful in tracing point-source contamination within the stormwater drainage network. Volumes of runoff water from specific storm events, calculated using the CN method, can be used to gauge the effects semi-permeable land uses have on the quantity and quality of runoff transported to surface waterbodies.

Designing a stormwater runoff prediction model that includes both the water flow direction and quantity of water transported is essential for not only urban stormwater management planners, but also for city utility officials and urban developers. In Northwest Arkansas, larger-scale replicates of these prediction models could play a crucial role in improving and preserving the quality of surface waterbodies like the West Fork tributary. With the advent of cost-effective monitoring programs, BMP construction, and education to improve water quality, the West Fork of the White River could eventually be removed from the impaired waterbodies list and have its biological productivity return to normal.

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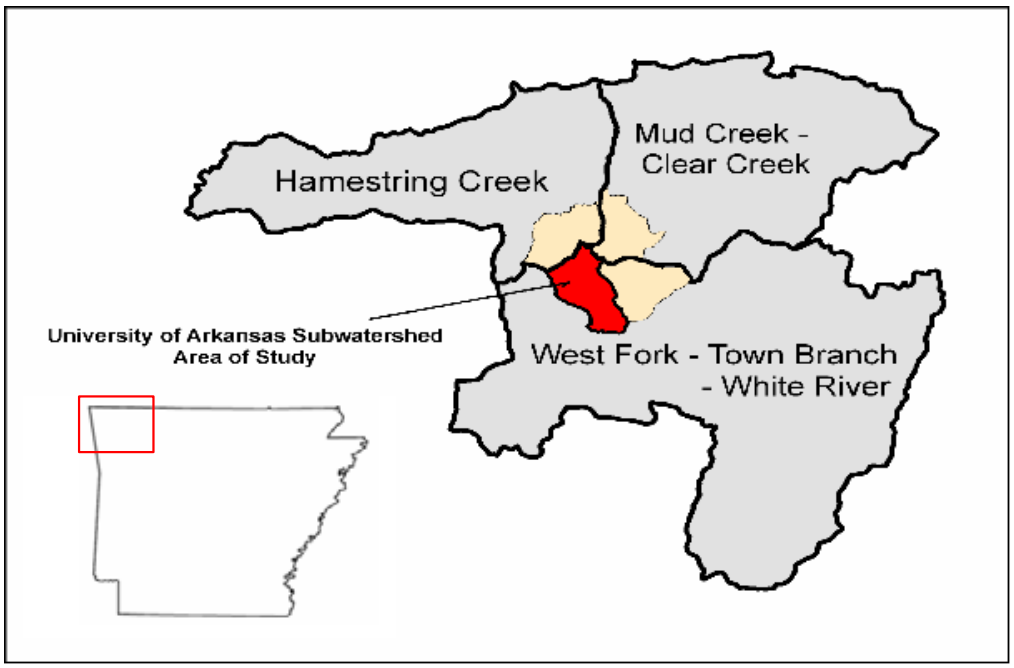

Fig. 1. Delineated University of Arkansas sub-watershed with surrounding NRCS HUC 12-Digit watershed boundaries. The study area was delineated from the West Fork – Town Branch – White River Watershed.

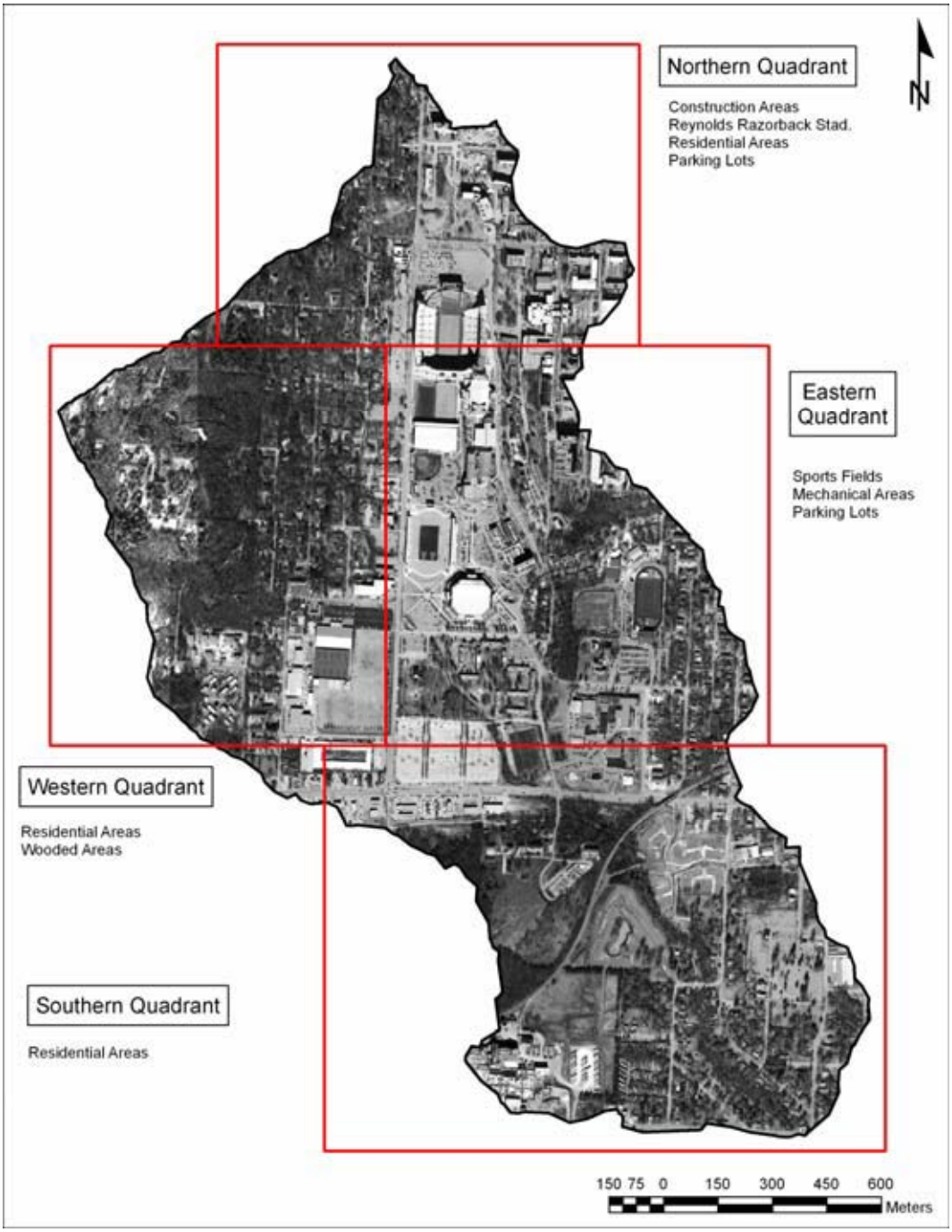

Fig. 2. Land-use quadrants within the study area are characterized by potential sources of runoff contamination. These sources include parking lots, construction zones, residential areas, and athletic fields.

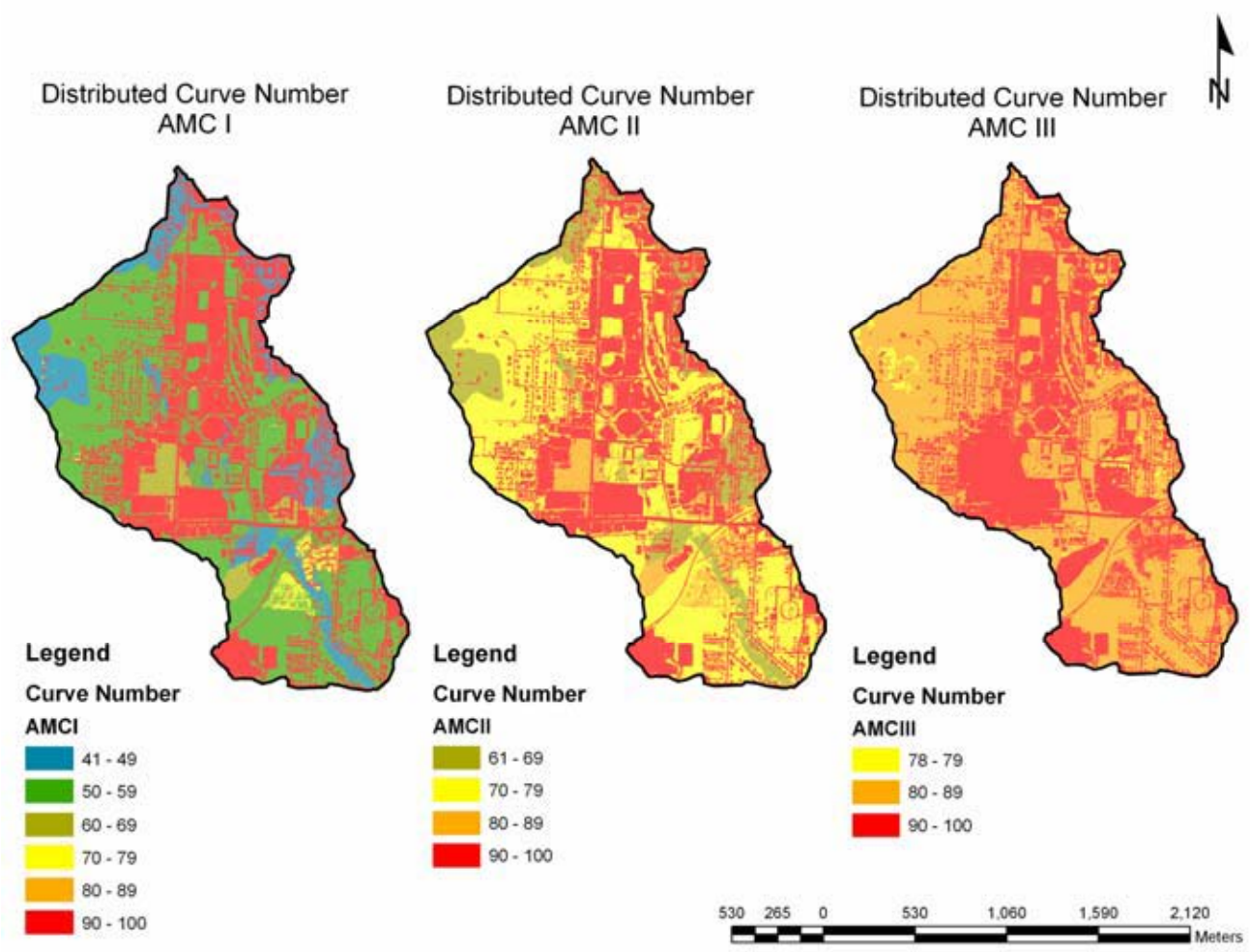

Fig. 3. Study area curve number analysis varied by AMC I, II, and III conditions. For each figure, the AMC was increased, increasing the spatially distributed CN and runoff depths.

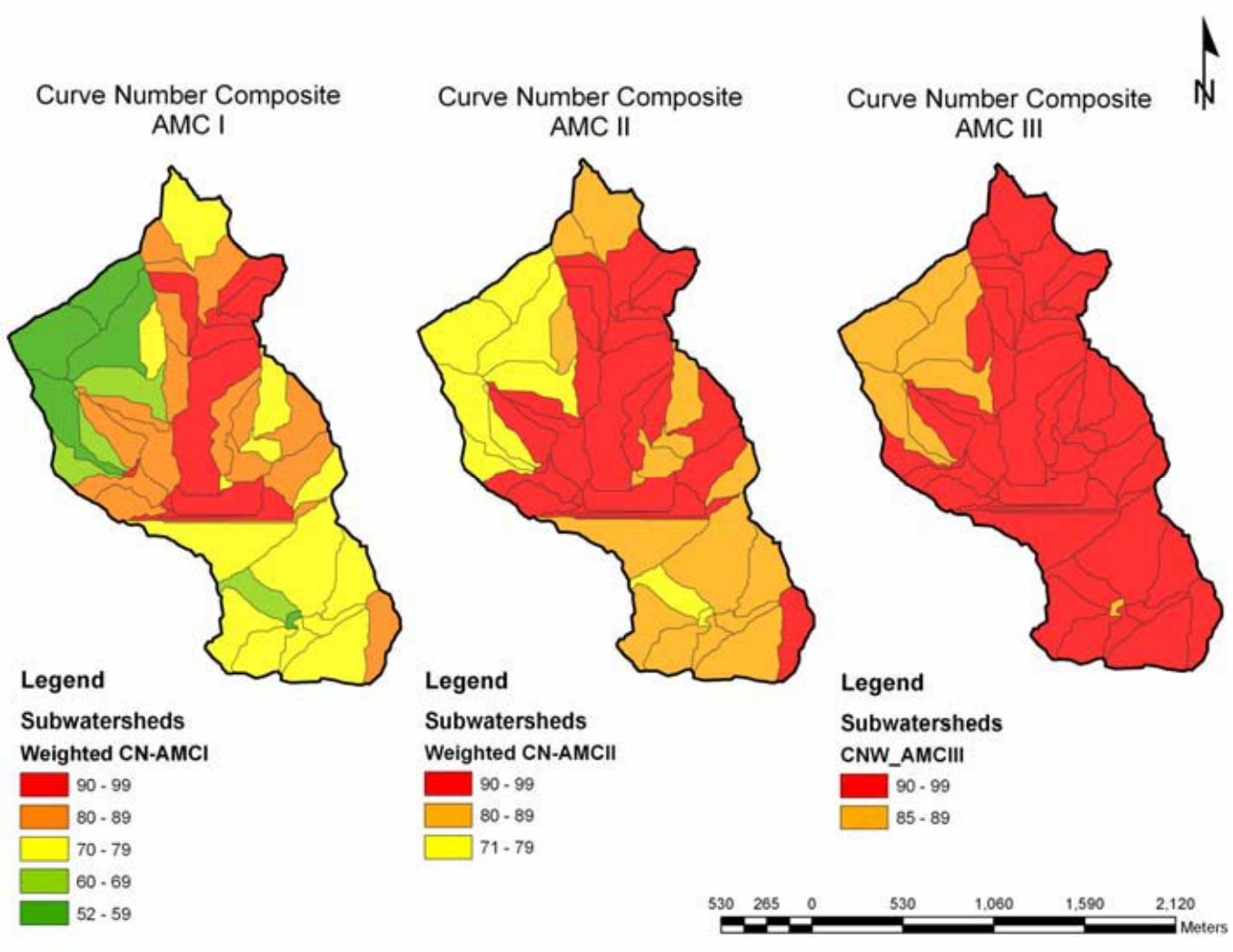

Fig. 4. Sub-watershed weighted curve numbers (CN) for varied AMC levels. For each map, left to right, the AMC was increased, thus increasing the average sub-watershed CN and runoff depths.

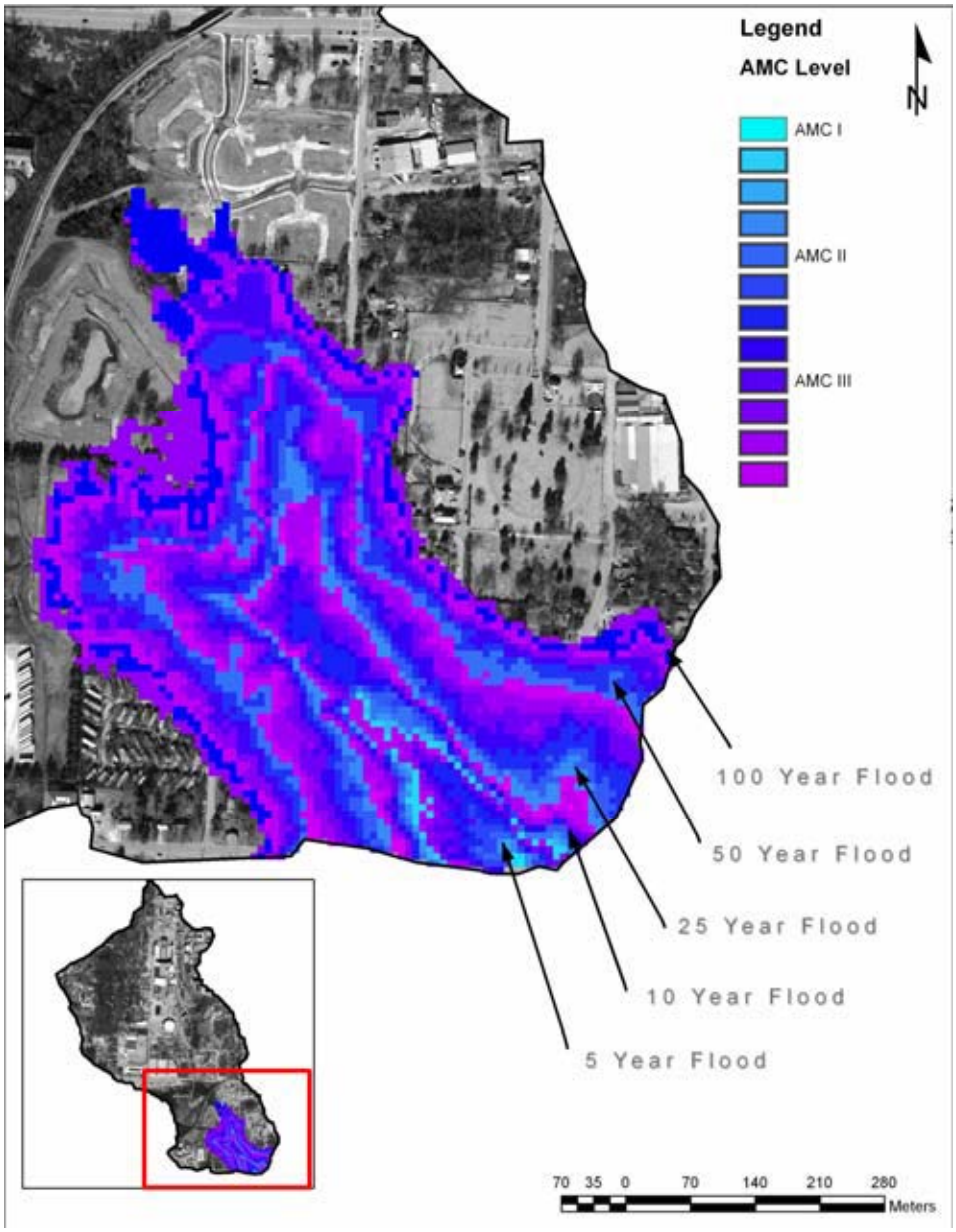

Fig. 5. Maximum flooding extent for varied AMC levels showing the locations of potential flooding on aerial photography of the southern quadrant of the study area.

Fig. 6. Picture of southern reach of College Branch Creek showing significant soil erosion.

Table **1**. Natural **R**esource Conservation Service hydrologic soils group (**HSG**) classification descriptions (**USD**A, **2007**)

HSG	Description of Classification
A	Soils having a high infiltration rate (low runoff potential) when thoroughly wet. These consist mainly of deep, well drained to excessively drained sands or gravelly sands. These soils have a high rate of water transmission.
B	Soils having a moderate infiltration rate when thoroughly wet. These consist chiefly of moderately deep or deep, moderately well drained or well drained soils that have moderately fine texture to moderately coarse texture. These soils have a moderate rate of water transmission.
C	Soils having a slow infiltration rate when thoroughly wet. These consist chiefly of soils having a layer that impedes the downward movement of water or soils of moderately fine texture or fine texture. These soils have a slow rate of water transmission.
D	Soils having a very slow infiltration rate (high runoff potential) when thoroughly wet. These consist chiefly of clays that have high shrink-swell potential, soils that have a permanent high water table, soils that have a claypan or clay layer at or near the surface, and soils that are shallow over nearly impervious material. These soils have a very slow rate of water transmission.

Table 2. Statistical information for the net runoff from the sub-watersheds based on curve number calculations

Statistic/ AMC level	5-yr Flood	10-yr Flood	25-yr Flood	50-yr Flood	100-yr Flood
Minimum			- mm -		
AMC I	28	36	51	63	77
AMC II	66	79	100	116	135
AMC III	100	115	139	158	179
Maximum					
AMC I	122	138	163	182	203
AMC II	136	153	178	197	219
AMC III	139	155	181	200	221
Mean					
AMC I	86	100	123	140	160
AMC II	110	126	150	169	190
AMC III	126	142	167	186	208

Table 3. Cumulative runoff depths and volumes for study area calculated by combining GIS calculations with the curve number method

Variable	5-yr Flood	10-yr Flood	25-yr Flood	50-yr Flood	100-yr Flood
Depth			- m -		
AMC I	4313	5031	6159	7021	8011
AMC II	5509	6297	7520	8443	9495
AMC III	6289	7105	8363	9308	10381
Volume			- m^3 -		
AMC I	24758	28975	35625	40716	46571
AMC II	32167	36851	44127	49627	55893
AMC III	37210	42083	49602	55252	61668

Impact of stressing a pen mate on physiological responses of growing pigs

Brent Koonce[*], *Beth Kegley*[†], *Doug Galloway*[§], *and Jason Apple*[‡]

ABSTRACT

Crossbred barrows and gilts (n = 36), weighing 16.59 ± 2.1 kg, were used to test the effects of stressing a pen mate on the physiological responses of growing pigs. Pigs were randomly allotted to 6 groups after stratifying according to gender, litter origin, and body weight. Dominance order was determined within each group, and 1 to 3 d prior to the stress treatment the most- and least-dominant pigs within a group were fitted with indwelling catheters in their vena cavas. Over 3 d, groups were either: 1) isolated from audile and visual contact with stressed pigs in a separate room (non-stressed control); 2) separated by a curtain from visual contact with stressed pigs; or 3) allowed to maintain audile and visual contact with stressed pigs. Blood samples were collected 30, 15, and 0 min before exposure to the stressor (snout-snare) treatment and again at 1, 2, 3, 4, 5, 7.5, 10, 15, 20, 25, and 30 min after stressor application. Serum cortisol and plasma glucose, lactate, and nonesterified fatty acids (NEFA) concentrations were measured. There were no treatment × sampling-time interactions ($P > 0.17$) for concentrations of cortisol, glucose, lactate or NEFA, nor were these metabolites affected by stressor treatment ($P > 0.42$). Humoral measures of the stress response were not affected by visual and/or audile contact with pen mates undergoing a stressful event.

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[†] Elizabeth B. Kegley is a professor in the Department of Animal Science and is the mentor for the project.

[§] Doug Galloway is a program associate in the Department of Animal Science.

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INTRODUCTION

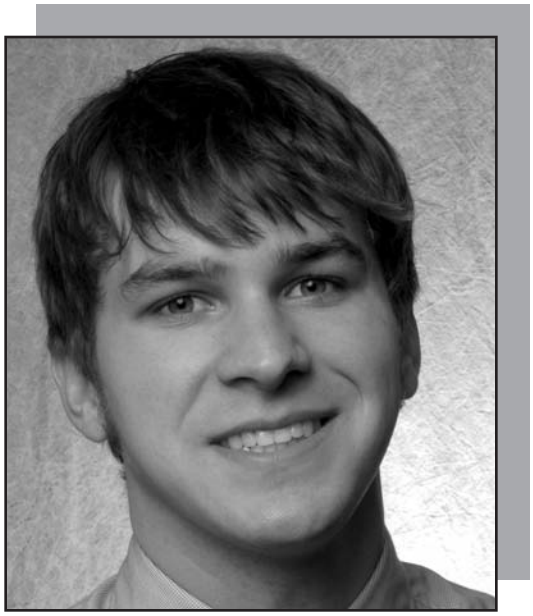
A problem facing the swine industry today is the amount of stress that pigs encounter throughout their lives. This stress may be caused by many factors including overcrowding, separation from cohorts, or any number of environmental factors such as heat stress. While this may seem to be a trivial matter, it is known that stress dramatically impacts a pig's nutritional intake and growth rate (Wellock et al., 2003). Stress may cause prenatal problems in swine if sows are stressed during gestation (Kanitz et al., 2003). Low birth weight and increased death rates during the preweaning period are just a few problems associated with this prenatal stress (Kranendonk et al., 2005). Therefore, swine producers should be conscious of stressors affecting their animals in order to maximize profitability.

More research in this area would benefit swine owners by possibly providing scientific evidence that pigs do in fact stress over other pigs' well-being status. This evidence would then further encourage swine producers to minimize their swine's stress levels not only for the animals' sakes, but also for their own. Reduced stress levels in the pigs would improve feed utilization, and the pigs would be more likely to reach their full potential for growth and efficiency and thus be more profitable for the producer.

The purpose of our research was to determine how indirect stress (stress of a fellow pen mate) affects pigs. The stress status of the pigs was measured by blood metabolite levels. Cortisol, shown to drastically increase during stressful situations, was one of the classic stress-indicating hormones (Widmaier, 2006) that was measured. Other blood metabolite levels that were monitored included lactate, glucose, and non-esterified fatty acids, all of which change in response to the amount of stress an animal experiences. Therefore, the objective of this study was to determine effects of indirect stress on pigs.

MATERIALS AND METHODS

During this 25 d study, the project utilized 36, 35 to 40 d old pigs (approximately 20 kg, 18 barrows, and 18 gilts), from the University of Arkansas Division of Agriculture swine herd. Pigs were blocked by gender, then divided into 6 groups of 6 pigs after stratification for litter origin and weight (3 groups of barrows and 3 groups of gilts). Pigs were housed in an off-site nursery facility with 2 separate rooms (Fig. 1). In one of the rooms, a curtain was hung dividing it into 2 equal sections, thus producing 3 visually isolated areas among 2 rooms. Two pens (4.2 × 1.2 m) in each of the 3 areas were used for housing the 6 groups, within each area



Brent Koonce

MEET THE STUDENT-AUTHOR

After graduating from Catholic High School (Little Rock, Ark.) in 2003, I began my college career at the University of California, Santa Barbara. However, after only a couple of semesters, my affinity for the Razorbacks and Arkansas' outdoors was too much to resist. In 2004 I enrolled in the Dale Bumpers College and the Honors College at the University of Arkansas as an Animal Science major.

Since joining the Animal Science program my sophomore year, I have become an active member in the Pre-Veterinarian Club, worked as an Animal Lab technician for the University of Arkansas' Central Laboratory Animal Facility, received the Alumni Society Scholarship, and completed an undergraduate research project funded by the Dale Bumpers College of Agricultural, Food and Life Sciences, as well as by an Honors College Undergraduate Research Grant. After college I plan on attending veterinary school to pursue my passion for the field.

None of the achievements above could have been possible without the help of my mentor, advisor, and friend, Dr. Beth Kegley. Also, my appreciation is given to Doug Galloway, who guided me throughout my entire research trial.

there was always one group of each gender. These large pens had completely slatted floors and were equipped with a 5-hole nursery feeder and 4 nipple waterers. From d 1 to 25, pigs had ad libitum access to water and typical grower diet (Table 1) that was formulated to meet all current NRC (1998) requirements for growing swine.

Every third day, groups were rotated among pens in order to become acclimated to human contact and pen rotation. Additionally, dominance order was determined within the groups by using an index of displacements equation (number of times a pig displaced another pig/(number of times a pig displaced another pig + number of times a pig was displaced)). Pigs were monitored for 30 min/d, on d 8, 9, 10, 14, 15, and 16. After social rank was determined, the most and least dominant pigs in each group were chosen for cannulation, and the middle-ranked pigs were used for snout snaring (the stressor).

On d 20 and 21, 2 pigs/group were fitted with cannulas in the vena cava. Pigs were anesthetized with isoflurane and nitrous oxide gas before being implanted with 90 cm of Tygon Microbore tubing (i.d. 1.27 mm). Sodium citrate was infused into the tubing, acting as an anticoagulant. Cannulated pigs were then housed individually after surgery but were returned to their respective areas (Fig. 1). Although now separated from original pen mates by a panel with vertical bars 5 cm apart, pigs were allowed visual and tactile (snout) contact with their cohorts.

On d 23, 24, and 25, the stressor treatments imposed were: 1) no contact with snared pigs (control), 2) audile contact with snared pigs, or 3) audile and visual contact with snared pigs. On these days, blood samples were taken from the cannulated pigs at -30, -15, and 0 min before snaring of their cohorts. Then, starting immediately after time 0, 2 pigs (one/pen) in the audile and visual treatment area (Area A, Fig. 1) were snared for a duration of 2 min. Blood samples were taken from the cannulated pigs at 1, 2, 3, 4, 5, 7.5, 10, 15, 20, 25, and 30 min after snaring began. At each sampling time, 4 ml of blood were drawn and then split and stored in plain tubes (1 ml) for serum collection and in tubes containing sodium fluoride (3 ml) for collection of plasma. Samples were stored on ice prior to centrifugation at 2,100 × g for 20 min for separation of serum or plasma. Serum was used to analyze cortisol concentrations by radioimmunoassay (DPC, Los Angeles, Calif.). Plasma was analyzed for glucose, NEFA, and lactate concentrations with colorimetric procedures.

This experiment was arranged as 3 × 3 Latin square design, replicated within gender, in which all groups were exposed to all 3 treatments over the 3 d. Data were analyzed using the PROC MIXED in SAS. The repeated statement was used with the SP(POW) covariance structure. The subject was group within day, whereas the experimental unit was group. Fixed effects were gender, treatment, day, replicate, gender × treatment, time, and time × treatment. Means were separated with an F-protected t-test.

RESULTS AND DISCUSSION

There was no treatment × sampling-time interaction (P = 0.55) for concentrations of cortisol (Fig. 2). Additionally, cortisol concentrations were not affected by stress treatment (P = 0.43) or gender (P = 0.57). Regardless of treatment, cortisol concentrations were greater 10 min after stress treatment began and lowest 1, 4, 5, 25, and 30 min after stress treatment began (time effect, P = 0.03). Also, serum cortisol levels tended to be greater (P = 0.10) on d 2 vs. d 1 of the experiment with d 3 being intermediate (data not shown). Cortisol, shown to dramatically increase in response to a stressor, is the classic stress-indicating hormone (Widmaier, 2006). Stress events such as regrouping pigs cause increased concentrations of cortisol (Coutellier et al., 2007). In the current study, no samples contained cortisol concentrations that would indicate high levels of stress; thus, the vocalizations and/or sight of a snared cohort did not elicit a classic stress response in other pigs.

Apple et al. (2005) reported an increase in glucose concentrations when finishing pigs were exposed to a stressor (transportation). Therefore, increased concentrations of plasma glucose would be expected in stressed animals due to mobilization of glycogen (Prunier et al., 2005). In this study, there was no treatment × sampling time interaction (P = 0.46) for circulating concentrations of glucose (Fig. 3). Furthermore, glucose concentrations were neither affected by stress treatment (P = 0.97) nor gender (P = 0.78), and plasma glucose concentrations were similar (P = 0.70) before and during stressor treatment. Additionally, plasma glucose levels did not (P = 0.62) differ across the 3 d of stressor treatments.

There were no main effects of stressor treatment (P = 0.91) or gender (P = 0.48), nor was there a treatment × sampling time interaction (P = 0.17) on plasma NEFA concentrations (Fig. 4). Plasma NEFA concentrations were not (P = 0.55) different before or during stressor treatment; however, NEFA concentrations measured on d 3 were greater (P = 0.01) than concentrations measured on d 1 and 2 of the stressor treatments (107 vs. 92, and 97 mmol/L for d 3, 1, and 2, respectively). In previously reported research with pigs (Apple et al., 2005), plasma NEFA levels decreased dramatically during the first 30 min of transportation, but plasma NEFA concentrations increased to levels greater than pre-transit levels

after 2 h of transportation. This is due to increased lipolysis and may explain the increase observed on d 3 in the cannulated pigs in the current study. Yet, absence of an increase in the plasma NEFA concentrations during stressor treatment would indicate there was not a stress response due to snaring the cohort.

Plasma lactate concentrations (Fig. 5) had no treatment × time interaction (P = 0.96) and were not affected by stress treatment (P = 0.60). However, barrows had greater (P = 0.003) plasma lactate concentrations than gilts. Plasma lactate concentrations were comparable before and during stressor treatment (P = 0.75). An increase in plasma lactate in monogastrics is related to catecholamine-initiated glycogenolysis and is another response to a stressor (Apple et al., 2005), but the lack of differences in plasma lactate concentrations is further support that study pigs did not respond to their cohorts exposed to a stressor.

In summary, swine undergoing stressful events have been shown to have reduced performance and produce poor meat quality. Knowing what causes stress would help reduce stress. Results of the current study suggest that humoral measures of the stress response are not affected by visual and/or audile contact with pen mates undergoing a stressful event.

ACKNOWLEDGMENTS

Financial support for this project was provided by the University of Arkansas Honors College and a Dale Bumpers College of Agricultural, Food and Life Sciences Undergraduate Research Grant. Also, the assistance of Matt Akins, Karen Anschutz, Clay Bailey, Benjamin Bass, Shollie Behrends, Lucy Bowman, Casey Bradley, James Caldwell, Tyler Davis, Dawn Elkins, Jason Frank, Mike Freyaldenhoven, Carlee Jamison, Linda Jones, Alex Kelch, Michele Lee, Becky Lockhart, Robin Ogden, Michael Person, Jeremy Powell, Sarah Sears, Jenny Thurlow, and Janeal Yancey is greatly appreciated.

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Table 1. Diet composition (as fed basis)

Item	%
Yellow corn	65.95
Soybean meal, 48% CP	28.86
Fat, yellow grease	2.30
Ethoxyquin	0.03
Lysine HCL	0.15
Threonine	0.02
Methionine	0.02
Tylan-40	0.05
Mineral premix[1]	0.10
Vitamin premix[2]	0.15
Monocalcium Phosphate	0.75
Calcium carbonate	0.92
Salt	0.50

[1] Mineral levels meet or exceed NRC (1998) recommendations
[2] Vitamin levels meet or exceed NRC (1998) recommendations

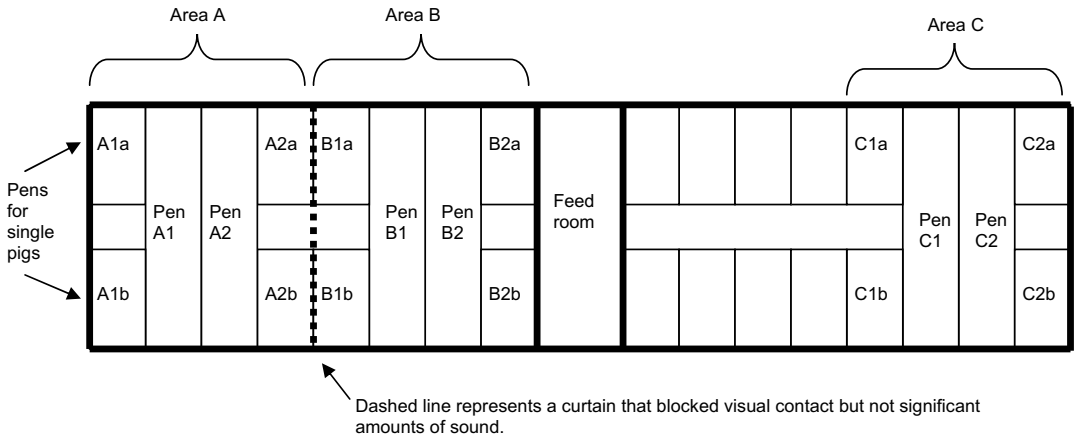

Fig. 1. Off-site nursery design (not to scale)

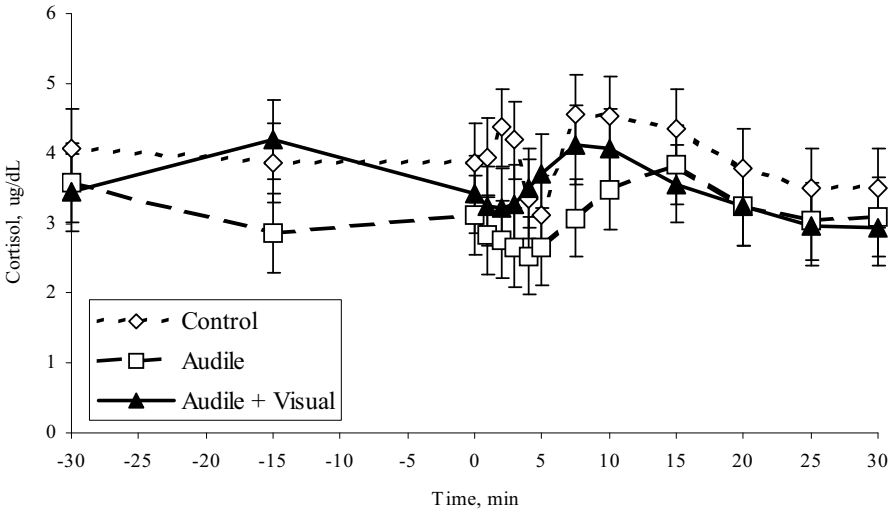

Fig. 2. Serum cortisol concentrations before and after stress treatment (stress treatment × time interaction, $P = 0.55$).

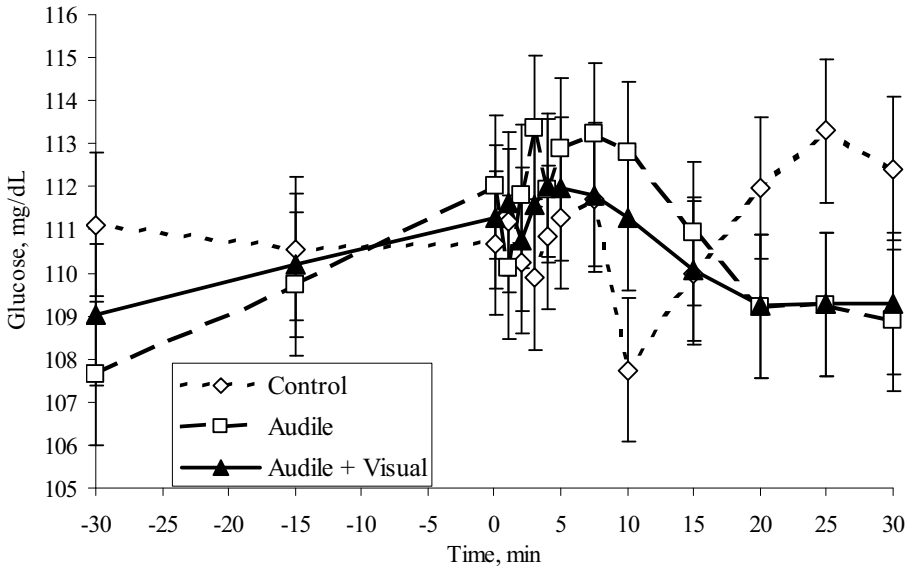

Fig. 3. Plasma glucose concentrations before and after stress treatment (stress treatment × time interaction, $P = 0.46$).

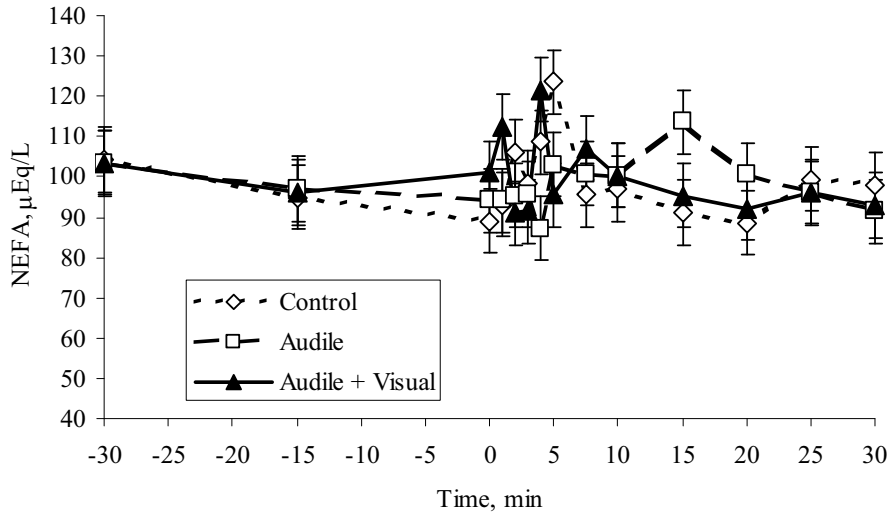

Fig. 4. *P*lasma NEFA concentrations before and after stress treatment (stress treatment × time interaction, *P* = 0.17).

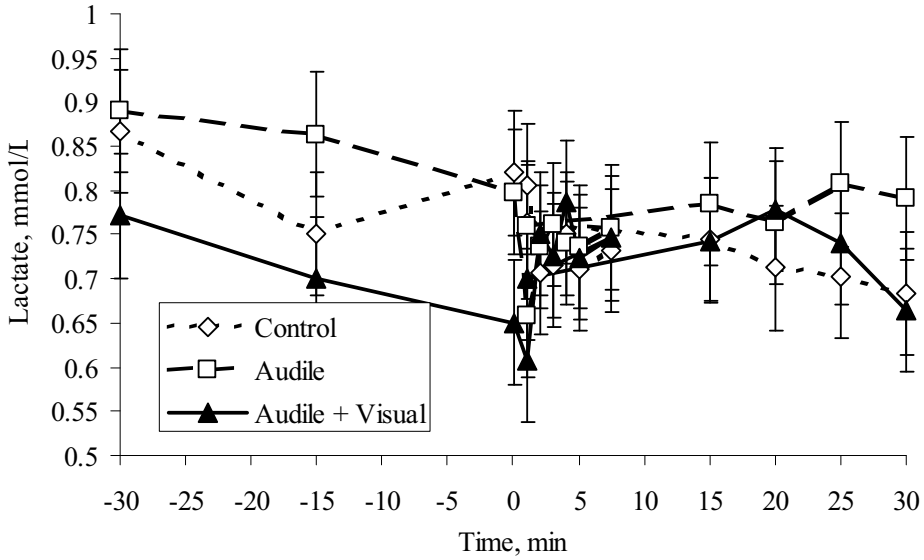

Fig. 5. Plasma lactate concentrations before and after stress treatment (stress treatment × time interaction, P = 0.96).

Using biosolids to enhance phytoremediation of oil-contaminated soil

Heather N. Markway[*], *Duane C. Wolf*[†], *Kaaron J. Davis*[§], *and Edward E. Gbur*[‡]

ABSTRACT

While the plant rhizosphere and associated microbial processes have been shown to amplify the degradation rate of chemical residues in soils, phytoremediation can be a slow process. The objective of this greenhouse study was to determine if the addition of biosolids as an organic soil amendment would enhance growth of plants in oil-contaminated soil and thus potentially increase effectiveness of phytoremediation. Pearl millet (*Pennisetum glaucum* (L.) R. Br.) or sudangrass (*Sorghum sudanense* (Piper) Stapf (Piper)) was grown in a Captina silt loam (fine-silty, siliceous, mesic Typic Fragiudults) contaminated with 5% crude oil (v/w) and amended with 24 g biosolids/kg soil. Addition of biosolids enhanced oil degradation after 10 weeks as indicated by the lower carbon (C) content in the oil-contaminated soil that was amended with biosolids compared to the C content of the oil-contaminated soil only. The addition of biosolids to the oil-contaminated soil resulted in a significant increase in plant shoot biomass. Pearl millet plus biosolids produced more root biomass, root length, root surface area, and root diameter than sudangrass plus biosolids in the oil-contaminated soil. The addition of biosolids also increased the amount of nitrogen (N) and phosphorus (P) in the soil. The results suggest that the addition of biosolids could increase potential for remediation of oil-contaminated soil.

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[†] Duane C. Wolf is a university professor in the Department of Crop, Soil, and Environmental Sciences.

[§] Kaaron J. Davis is a program associate in the Department of Crop, Soil, and Environmental Sciences.

[‡] Edward E. Gbur is a professor and interim director of the Agricultural Statistics Laboratory.

Heather Markway

MEET THE STUDENT-AUTHOR

I am from Saddle, Ark., but graduated from the Arkansas School for Mathematics, Sciences and the Arts in Hot Springs in May 2003. That fall I enrolled in the University of Arkansas as an undecided major in the Fulbright College of Arts and Sciences. In fall 2004 I declared my major in environmental, soil and water sciences in the Department of Crop, Soil, and Environmental Sciences and joined the AFLS Honors Program.

As part of my honors program requirements, I worked with my honors mentor, Dr. Duane Wolf, on a greenhouse project to evaluate the use of biosolids for phytoremediation. My research project was funded by the U of A Honors College as well as by the Carroll Walls AFLS Undergraduate Research Grant through the Dale Bumpers College of Agricultural, Food and Life Sciences.

After completing my honors research project, being able to study for a semester at the Scottish Agricultural College in Edinburgh, Scotland, and getting to participate in the Belize Service Project (all as part of my B.S. degree), I graduated in August 2007. I look forward to continuing my studies in graduate school and eventually working as an environmental liaison between developing and developed countries.

INTRODUCTION

Terrestrial oil spills cause many problems within ecosystems due to environmental and health issues posed by oil-contaminated soil (Cunningham et al., 1996). Oil contamination comes from many sources, including tankers, holding tanks, oil-water separators, dissolved air floatation units, and drilling operations (Manning and Thompson, 1995). According to Anderson et al. (1993), oil spills adversely impact the environment in multiple dimensions. Biologically, oil can be detrimental to both plant and microbial life present in the area of a spill. At high oil concentration, most, if not all, plants originally in the area of an oil spill die. Chemically, numerous organic compounds including polycyclic aromatic hydrocarbons (PAHs) increase in oil-contaminated soil and can reduce plant growth (Baker, 1970). Physically, since oil is hydrophobic, petroleum creates a water-impermeable layer in the soil.

Common soil remediation options for oil-contaminated soils are excavating soil and either hauling it to a landfill or to an incinerator. Both options are costly but are common techniques in areas where it is important to clean up the contaminated site quickly because of human health and land-use concerns (Ward et al., 2003). For spills that occur in more remote areas where money is a more important commodity than time and space, it is desirable to find an economically and environmentally acceptable way of remediating contaminated soils.

Phytoremediation is defined as the use of green plants to remove, contain, or render harmless environmental contaminants (Cunningham and Lee, 1995). Plant rhizospheres, plant roots in conjunction with their associated microbial communities, have been shown to amplify the microbial degradation rate of chemical residues in soils (Anderson et al., 1993). Growing plants in contaminated soil can be a cheaper alternative or a supplement to more expensive soil remediation options. Phytoremediation costs to clean up oil-contaminated soil have been estimated at $162/m3 as compared to removal and incineration at an estimated $810/$m^3$ (Rock and Sayre, 1998). Phytoremediation can be not only cost-effective but also low-maintenance and environmentally friendly (Cunningham et al., 1996). While phytoremediation is much less costly than traditional remediation, it is also a slow process. In some cases it takes years for plants and their associated microorganisms to degrade contaminants to a safe level (Boopathy, 2000). Therefore it is important to develop strategies to speed up the degradation process.

Grasses with their fibrous root systems can support greater microbial numbers and activity than taproot

plants (Anderson et al., 1993), and therefore have been used in many phytoremediation projects (White et al., 2003; Dickinson and Rutherford, 2006). Addition of organic amendments such as poultry litter, inorganic fertilizer, hardwood sawdust, or biosolids has been found to enhance plant growth in oil contaminated-soil (White et al., 2003). Biosolids are nutrient-rich organic material resulting from the treatment of wastewater and are commonly used as agricultural amendments to increase plant growth (EPA, 2007). Due to net N mineralization of organic-N, biosolids provide plants with a steady supply of N over the growing season. Increased root growth would increase potential rhizosphere microbial activity, resulting in a higher rate of oil degradation and more effective phytoremediation. Biosolids also improve soil structure by decreasing soil bulk density and increasing porosity and thus increasing the ability of soil to absorb and hold water (EPA, 2007; Dickinson and Rutherford, 2006).

Juteau et al. (2003) found that addition of biosolids to non-vegetated soils enhanced degradation of alkanes. Dickinson and Rutherford (2006) used biosolids to enhance degradation of diesel hydrocarbons in contaminated soils; they concluded that addition of biosolids to contaminated soil increased the soil and plant N contents. Soil physical and chemical properties such as water-holding capacity, cation-exchange capacity, and pH were also increased.

The objective of the 10-week greenhouse study was to determine the influence of biosolids addition on pearl millet or sudangrass growth and soil chemical properties in crude oil-contaminated soil.

MATERIALS AND METHODS

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) or sudangrass (*Sorghum sudanense* (Piper) Stapf (Piper)) was grown in oil-contaminated soil 1) with treatments of biosolids or 2) unamended to determine the influence of biosolids addition on plant growth. A non-vegetated treatment was also included. There were two treatments (biosolids or unamended), three vegetations (pearl millet, sudangrass, or no plant), and four replications, for a total of 24 individual sample units.

Soil. Soil used for the study was a Captina silt loam (fine-silty, siliceous, mesic Typic Fragiudults) collected from the Arkansas Agricultural Research and Extension Center, Fayetteville, and passed through a 2-mm stainless-steel sieve. On a dry-weight basis, 500 g of Captina silt loam soil (0.97% total C) were contaminated to 5% crude oil (v/w) with 25 mL (22.4 g) of crude oil collected from a drilling site near El Dorado, Ark. Oil and soil were thoroughly mixed by hand in plastic bags. After allowing the bags to sit for four days, the amount of oil volatilization was measured by reweighing the bags, and a mean of 3.0 g of volatile organic compounds were lost. The biosolids were aerobically digested sewage sludge obtained from the Springdale Wastewater Facility and analyzed by the Arkansas Agricultural Diagnostics Laboratory (Table 1). Assuming 85% C in crude oil, the amount of C remaining in the soil following volatilization losses was 33g C/kg soil. To attain a 20:1 C:N ratio for optimal microbial activity, 11.8 g biosolids dry weight/pot were added based upon the necessary addition of 1.65 g N/kg soil. The wet biosolids were weighed and mixed into the appropriate soil-oil mixture bags. The soil-oil amendment mixture was transferred into a Cone-tainer® (Stuewe & Sons, Inc., Corvallis, Ore.) (25-cm high x 6.4 cm in diameter) and the soil was adjusted to a water potential of –33 kPa (17.5% Θ_w).

Plants. The sample units for this experiment were placed in a randomized complete block design and grown in the University of Arkansas Greenhouse 3.2 for 10 wks. Ten seeds of pearl millet or sudangrass were planted at a depth of approximately 1 cm in the appropriate Cone-tainers®, except for the non-vegetated samples. At 2 wks, the plants were thinned to 3 plants/pot, and at 3 wks, plants were thinned to 1 plant/pot. Soil moisture was maintained by daily watering with deionized water.

Sample processing. After 10 wks, the plants were harvested by cutting the shoots at the soil surface, rinsed with deionized water, dried to a constant weight at 65°C, and weighed to determine shoot biomass. The roots were separated from the soil by gently shaking the soil cores onto a tinfoil-lined tray, breaking the soil up, and using tweezers to remove the roots. The roots were then rinsed with deionized water on a 500 μm stainless-steel sieve and stained with a solution containing 0.1 g methylene blue/L 10% ethanol. After letting the stain set overnight, the staining solution was discarded and the roots were placed in a layer of water in the scanning dish of the WinRHIZO Digital Imagery System® (Regent Instruments, Inc., Quebec, Canada). The roots were scanned by the imagery system, and the root length, average diameter, and surface area for each sample were determined from the image and an associated computer program (White et al., 2003). After scanning, root biomass was determined by drying roots to a constant weight at 65°C and weighing.

Soil samples were sent to the Arkansas Agricultural Diagnostics Laboratory to determine the Mehlich 3-extractable nutrient contents and total C and N levels. Semi-micro Kjeldahl steam distillation was used to determine the NH_4-N and NO_3-N concentrations in the soils (Keeney and Nelson, 1982).

Statistical analyses were completed by the Agricultural Statistics Laboratory of the University of Arkansas using the GLM Procedure with the means separated by the LSD at $P \leq 0.05$ using SAS® software, version 9 (SAS Institute, Cary, N.C.).

RESULTS AND DISCUSSION

Influence of biosolids on plant growth. With the addition of biosolids to the oil-contaminated soil, shoot biomass production after 10 wks was significantly greater than in the oil-contaminated soil with no amendment, with mean values of 2.59 and 0.02 g/plant, respectively. There was not a difference between pearl millet and sudangrass shoot production. The addition of biosolids resulted in a significant plant species-by-amendment interaction with the greatest root biomass, root length, root surface area, and mean root diameter for pearl millet grown in the biosolids-amended oil-contaminated soil (Table 2). Pearl millet and sudangrass grown in the unamended oil-contaminated soil exhibited minimal growth and were not different for the four parameters evaluated.

Addition of biosolids to the oil-contaminated soil stimulated plant growth with the increase in soil N and P levels. Oil-contaminated soils generally have low concentrations of soil N because of net immobilization by microorganisms breaking down the C from the contaminant (Xu et al., 1995). When additional N is introduced into the system, plant growth increases. Pearl millet had a significantly larger root-growth response to the addition of the biosolids than did the sudangrass, indicating that the pearl millet would have a greater rhizosphere and thus could facilitate greater degradation of the contaminant. These findings are consistent with Kirkpatrick et al. (2006), who reported that pearl millet had significantly greater root length and surface area than sudangrass in oil-contaminated soil amended with 425 to 1275 mg N/kg soil.

Influence of biosolids on soil chemical properties. Total C in the crude oil-contaminated soil was significantly less in the treatments with added biosolids than in those without added biosolids, with values of 3.155% and 3.573%, respectively, regardless of vegetation treatment. Total N and NH_4-N contents were significantly higher in the soils amended with biosolids than in the non-amended samples, with the highest being the no plant-biosolids treatments, which were significantly higher than the vegetated treatments (Table 3). The NO_3-N concentration of the biosolids-amended pearl millet treatment was not significantly different from the non-amended treatments, while the sudangrass-biosolids treatment was significantly greater than the no amendment treatments (Table 3). The no plant-biosolids amendment treatment had the highest levels of NO_3-N. With addition of biosolids, the amounts of P, Ca, Zn, and Cu were significantly higher than in the unamended treatments regardless of vegetation treatment (Table 4).

The most important finding following the 10-week study was that C content of the biosolids-amended oil-contaminated soil was significantly less than oil-contaminated soil without biosolids. Even with the 4.2 g C/pot added with the biosolids, there was less C in the biosolids-amended treatments at the end of the 10-wk study, suggesting that N was limiting the degradation of oil in the unamended samples. The amount of C from the oil addition was the same across the experiment before the addition of biosolids to the amended samples. In order for the amended samples to have less C at the end of the study, the data show that the biosolids stimulated degradation of the oil with or without plants. In other studies, addition of organic amendments to oil-contaminated soils has been shown to decrease Total Petroleum Hydrocarbons (TPH) over time. White et al. (2003) found that addition of broiler litter resulted in reduction of gravimetric TPH levels across six plant treatments.

The high amount of total N, NH_4-N, and NO_3-N in the no plant-biosolids amended treatment indicated that N added to the system exceeded the microbial requirements and without plant uptake, the additional N remained in the soil. The NO_3-N levels in the pearl millet amended and all non-amended treatments were not significantly different, suggesting that the high levels of growth of the pearl millet in the biosolids-amended treatment used the NO_3-N as soon as it was produced. These results were consistent with findings by Dickinson and Rutherford (2006) where they tested use of biosolids during phytoremediation of hydrocarbon-contaminated soils. The sudangrass-amended treatments had significantly more NO_3-N than did the pearl millet because, with about half the root growth, the sudangrass would have utilized less NO_3-N for growth processes and rhizosphere activity.

Plants utilize both the NO_3-N and NH_4-N for growth processes. With available O_2 and CO_2, along with an abundance of Ca ions, the NH_4-N should have rapidly undergone nitrification, being quickly converted by *Nitrosomonas* sp. bacteria to NO_2-N and then converted by *Nitrobactor* sp. bacteria to NO_3-N. In most soils, NO_3-N is generally the predominant mineral form of N (Brady, 2002). However, the data show that high amounts of NH_4-N remained in the biosolids-amended soil, which indicated that the oil inhibited the first step of nitrification by *Nitrosomonas* sp. Plants significantly

decreased the amount of NH_4-N in the biosolids-amended soil compared to the soil of the no plant-biosolids treatment because NH_4-N was easily taken up by the plants for growth processes.

Biosolids addition to the soil resulted in increased levels of P, Ca, Zn, and Cu compared to the unamended treatments (Table 4). The addition of the essential nutrients for plant growth could also result in enhanced phytoremediation. The P was not significantly different between the biosolids-amended vegetative and non-vegetative treatments, suggesting that P was not limiting before the addition of biosolids. The increase in Zn and Cu with the addition of biosolids is noteworthy because of the concerns associated with the accumulation of trace elements in soils amended with organic waste materials such as biosolids. When considering use of biosolids as an amendment to an oil-contaminated site, the addition of trace elements might warrant additional consideration.

The C data indicated that addition of biosolids stimulated the degradation of crude oil in contaminated soil. Not surprisingly, the addition of biosolids increased the plant-available nutrients in the soil, including the NH_4-N, NO_3-N, P, and Ca, which, in turn, increased pearl millet and sudangrass growth as measured by shoot and root biomass, root length, root surface area, and mean root diameter. The high NH_4-N concentrations in biosolids-amended soil suggested that the oil was inhibiting the nitrification process. This study indicated that amending oil-contaminated soil with biosolids can enhance plant growth, which has the potential to increase the effectiveness of phytoremediation.

ACKNOWLEDGMENTS

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Table 1. The C and N concentrations in the aerobically digested biosolids used in the greenhouse study.

Parameter	Units	Concentration (dry wt.)
Total C	%	35.8[1]
Total N	%	7.0
C/N Ratio		5.1/1
NH_4-N	mg/kg	1,144
NO_3-N	mg/kg	166

[1] The values are a mean of two samples.

Table 2. Interaction of soil amendment and plant species on root biomass, length, surface area, and mean diameter in crude oil-contaminated soil following a 10-wk greenhouse study.

Soil Amendment	Plant Species	
	Pearl Millet	Sudangrass
	Root Biomass	
	------ g/plant ------	
Biosolids	0.90 a[1]	0.47 b
No Amendment	0.01 c	0.02 c
LSD = 0.27		
	Root Length	
	------ cm/plant ------	
Biosolids	5049 a	2151 b
No Amendment	111 c	162 c
LSD = 1427		
	Root Surface Area	
	------ cm^2/plant ------	
Biosolids	806 a	332 b
No Amendment	11 c	19 c
LSD = 259		
	Mean Root Diameter	
	--------mm--------	
Biosolids	1.24 a	0.74 b
No Amendment	0.32 c	0.38 c
LSD = 0.31		

[1] Means for a given plant parameter in the table followed by the same letter are not significantly different at $P \leq 0.05$

Table 3. Interaction of soil amendment and plant species on total N, NH_4-N, and NO_3-N in crude oil-contaminated soil following a 10-wk greenhouse study.

Soil Amendment	Vegetation Treatment: Pearl Millet	Sudangrass	No Plant
		Total N	
		------ % ------	
Biosolids	0.202 b[1]	0.212 b	0.226 a
No Amendment	0.096 c	0.096 c	0.093 c
LSD = 0.011			
		NH_4-N	
		------ μg N/g dry soil ------	
Biosolids	248.1 b	291.5 b	413.2 a
No Amendment	1.8 c	1.4 c	1.7 c
LSD = 72.7			
		NO_3-N	
		------ μg N/g dry soil ------	
Biosolids	19.1 c	83.3 b	156.5 a
No Amendment	0.9 c	0.7 c	1.5 c
LSD = 40.0			

[1] Means for a given N form followed by the same letter are not significantly different at P ≤ 0.05.

Table 4. Main effect of biosolids amendment on plant available P, Ca, Zn, and Cu concentrations in crude oil-contaminated soil following a 10-wk greenhouse study.

Soil Amendment	P	Ca	Zn	Cu
		------ mg/kg ------		
Biosolids	503.2 a[1]	979.3 a	5.5 a	1.6 a
No Amendment	26.9 b	278.3 b	0.8 b	0.5 b
LSD	29.1	69.6	0.4	0.1

[1] Means in column followed by the same letter are not significantly different at P ≤ 0.05.

The effect of Austrian winter-pea cover crop and cow-pea companion crop on corn yield

Matthew Marsh[*]*, David Longer*[†]*, and Vaughn Skinner*[§]

ABSTRACT

Leguminous cover crops have the potential to combat the rising input cost of commercial nitrogen (N) fertilizers. This experiment examines benefits of implementing a leguminous cover and/or companion crop into a corn production system. Legumes biologically fix nitrogen from the atmosphere, adding to the nitrogen content of the soil. In this experiment Austrian winter peas (*Pisum arvense*) (AWP) were used as the leguminous cover crop and cowpeas (*Vigna unguiculata*) were used as the companion crop. A two year experiment was carried out in which winter peas were planted on half the field in the fall and allowed to grow until late April to early May. The pea biomass was recorded, then the peas were plowed into the soil allowed to incorporate and begin decomposition, followed by corn planting. Different rates of commercial nitrogen were applied and varying seeding rates of companion-crop peas were also evaluated. Nitrogen was applied at 0, 112, and 224 kg ha^{-1}. Companion-crop peas were planted at 0, 4, and 8 plants m^{-1}. The corn was harvested, and yield as influenced by the various treatments, was evaluated. In both years, cover-crop peas provided all or a significant amount of corn N needs. This has useful implications for producer profitability and the environment since commercial N requires fossil fuels during its production.

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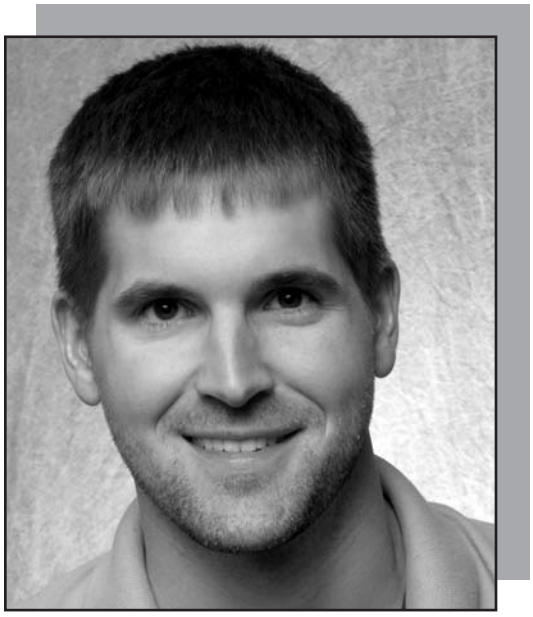
INTRODUCTION

Cost of nitrogen (N) fertilizers has increased greatly in recent years. This is due to ever increasing cost of fossil fuels needed to produce commercial nitrogen fertilizers. To combat increasing economical and environmental costs of production, producers need a highly productive and sustainable alternative. It is becoming necessary for producers to maintain or increase productivity, and increase sustainability. For farming systems to adapt to do this long term, it will be necessary to replenish reserves of nutrients that are removed or lost from the soil (Peoples et al., 1995(a).) One possible way to achieve this is through use of cover cropping. A cover crop is the planting of grass or legumes on a field between production seasons. Such plantings reduce erosion, build soil, prevent leaching, and in the case of legumes, fix nitrogen for subsequent crops (Sullivan et al., 2003.) Management practices consisting of combinations of conservation tillage, mixtures of legume and non-legume cover crops, and reduced rates of N fertilizers have potential for sustaining crop yields, increasing soil carbon (C) and N storage, and reducing soil N leaching, thereby helping improve soil and water qualities (Sainj et al., 2003.) Winter cover crops may also increase soil organic matter while sequestering N and preventing leaching into the groundwater. This is important for the low-organic matter alluvial soils of the Mississippi River Delta. Intensive cropping of this area for many years has caused depletion of organic matter and brought about an urgent the need for its conservation (Mascagni et al., 1997.)

Use of a leguminous cover crop contributes biologically fixed nitrogen from the atmosphere, with potential for reducing the N fertilizer requirement (Mascagni et al., 1998.) Leguminous companion crops, defined as legumes planted along with the target crop, can also be implemented to reduce the N fertilizer requirement (Davis et al., 1991.) Atmospheric nitrogen is fixed symbiotically by associations between *Rhizobium* sp bacteria and legumes. This relationship represents a renewable source of N for agriculture. Contributions of legume N_2 fixation to the N-economy of any ecosystem are mediated by legume reliance upon N_2 fixation for growth, the total amount of legume-N accumulated, and legume biomass (Peoples et al., 1995(b).)

Some previous research shows legume cover crops can supply all or most of the N required by a

MEET THE STUDENT-AUTHOR



Matthew Marsh

I have been involved in agriculture my entire life. My family has operated a farm near McCrory, Ark. for generations. This deep background has inspired my interest in agriculture at the University of Arkansas. After graduating from McCrory High School, I came to the University of Arkansas on an Arkansas Academic Challenge Scholarship. Throughout my career at the University I have been involved in many different organizations and events. I am a member of Kappa Sigma fraternity where I participated in many events with Greek life. I represented the Dale Bumpers College of Agricultural, Food and Life Sciences as a senator in Associated Student Government. I have also served as a peer mentor for incoming students of the Dale Bumpers College of Agricultural, Food and Life Sciences. To further my education outside the classroom, I interned with Monsanto Company throughout the summers of 2006 and 2007. This was an incredible experience that sparked my interest in research. I am graduating in May 2008 and plan on continuing my education here at the University of Arkansas with a master's degree in crop science.

target crop if legume biomass is of sufficient quantity and N mineralization is approximately synchronous with crop demand. In one such experiment, sweet corn following rye exhibited a linear response to N fertilizer (up to 156 kg N ha), but generally exhibited no response to added N fertilizer following legumes, alfalfa, or hairy vetch plus rye. In this experiment it was concluded that the legume cover crops grown were able to replace all or nearly all of the N fertilizer required by a subsequent sweet corn crop. These cover crops were found to be a viable alternative source of N, greatly reducing or eliminating the need for N fertilizer (Griffin et al., 2002)

In order to gain a better understanding rate of existing or emerging biological nitrogen fixation technologies, it has been proposed (Bantilan et al., 1995) that future research and development efforts focus on research adaptation and practical use. Giller et al. (1995) also stated that strategies enhancement and exploitation of biological nitrogen fixation be assessed with attention to the timescales for realization of benefits biological nitrogen fixation may have in agriculture. The experiment reported in this paper contributes to that objective. Immediate enhancements in inputs from N_2-fixation are possible by simple implementation of existing technical knowledge, such as cover cropping. Legume selection must be considered within the context of the farming system and geographical region in which specific legume species are grown. Proper integration of legumes requires a good understanding of the role of the legume within the system and a better understanding of the relative contributions of N sources and the fates of fixed N (Giller et al., 1995) We believe the experiment presented in these pages brings greater understanding of what leguminous cover crops can contribute to nitrogen requirements and replacement in corn. Our objectives in this study were to: 1) determine if all or a portion of seasonal N crop needs of corn could be met with cover and/or companion crops, and, 2) establish whether corn grown with cover crops can influence and perhaps improve the soil nutrient profile.

MATERIALS AND METHODS

The field experiments were conducted over the time period of October 2005 until October 2007 at the University of Arkansas Agricultural Experiment Station, Fayetteville, on a silt loam soil. The experimental design was a basic split plot. The whole-plot portion was a randomized complete block (RCB) with one factor; AWP with two levels: AWP and no AWP. The split plot portion was a RBC with 4 replications and 2 treatment combinations which were nested within each experiment unit of the whole plot. For analysis purposes it was assumed that blocks and replications were random effects and winter peas, cowpeas and nitrogen were fixed effects.

Treatments were arranged as follows: winter cover crop (AWP, no AWP); intercrop cowpeas (0, 6, and 13 plants m^{-1}); and nitrogen fertilizer rates (0, 112, and 224 kg ha^{-1}). The plots were four rows (100 cm) wide and 3 meters long.

In October 2005 and 2006 the field was cultivated and half was planted in Austrian winter peas (14 kg/ha). In mid-May 2006 and 2007, respectively, the peas were mowed and disked into the soil when dry. The peas had begun flowering when mowing occurred. The field was bedded in 1-m rows. The field was cultivated and planted on June 1, 2006 and June 10, 2007 with Pioneer 31G96 at 62,000 plants per ha^{-1}. The cowpeas were planted along with corn at previously mentioned seeding rates. Nitrogen treatments were applied to plots as split applications by hand-scattering N, with precise amounts applied to each plot. The plots receiving 112 kg of N received a single application approximately 10 d after planting. Plots receiving 224 kg ha^{-1} received 112 kg ha^{-1} 10 d after planting, with the second 112 kg ha^{-1} coming 30 d after planting.

Pea dry-matter yield was taken just before the AWP were mowed. Five one meter square areas of the AWP were harvested dried, and weighed. Weights were averaged and multiplied by the field area to determine the total dry-matter production. A portion of the dry matter was also analyzed for percent nitrogen content. Nitrogen percentage was multiplied by total dry-matter production to determine total nitrogen production by the pea aboveground dry matter.

Volumetric soil water content was measured in the 0- to 6-cm depth throughout the AWP cover-crop area and the non-cover-crop area just prior to pea harvesting. This was accomplished by using a Theta Probe (model TH20, Dynamax, Houston, Texas, USA), which records dielectric voltage readings and converts them to volumetric water contents using a soils-specific calibration equation.

Pesticide applications in 2006 were Duel Magnum, a selective herbicide labeled for most annual grasses and small-seeded broadleaf weeds formulated with *S-metolachlor* as the active ingredient, and Asana, an insecticide formulated with *esfenvalerate* as the active ingredient, to control a wide range of insects. The Duel Magnum application was made on June 9, 2006, one day prior to planting; it was applied at 1.56 liters ha^{-1}. Two Asana applications were made; the first being July 6 at .59 liters ha^{-1} and the second on July 20 at .73 liters ha^{-1}. Weeds were also controlled with single-row cultivation on July 6, 2006. In 2007 Duel Magnum was applied June 1, 2007

at 1.56 liters ha^{-1} prior to planting. Single-row cultivation was also used to control weeds in 2007. In 2007 no Asana application was made.

Irrigation was managed through row saturation from furrow irrigation, at the discretion of the farm manager. The water was applied via 15-cm aluminum pipe until furrows were saturated. Irrigation occurrences are shown in Table 1. In 2006 the field was irrigated 6 times and 4 times in 2007.

Entire corn ears were hand-harvested on October 12-13, 2006 and October 15-17, 2007. A 1.5-M portion of the middle two rows was harvested. The ears were bagged and dried to below 13% moisture. After drying, corn was shelled by hand using an electric sheller. Grain was then weighed and adjusted to 13% moisture. The data were analyzed by analysis of variance through (ANOVA) procedures. Statistically significant main effects and interactions are presented in the Results and Discussion section.

Irrigation data for 2006-2007 growing season. Days on which irrigation was applied to plots.

Dates	
-2006-	**-2007-**
July 7	July 31
July 20	August 8
July 28	August 14
August 4	August 22
August 9	
August 18	

Table 1 shows no statistical difference in 2006 between plots receiving 0, 112, and 224 kg ha^{-1} N when an AWP cover crop was present. Interestingly, plots receiving no N, with winter cover crop peas present, and plots receiving 112 kg ha^{-1} N, with no cover-crop present, yielded very similarly and actually produced greater yield than the plots receiving 224 kg ha^{-1}, with no pea cover crop, in 2006. These data imply that the winter-pea cover crop replaced as much N as delivered by the 224 kg ha^{-1} application of commercial fertilizer. Plots receiving no N, but with cover crop peas, produced a grain yield of 3230.4 kg ha^{-1}, which was significantly different ($p=0.05$) from the plots receiving no N and no cover-crop peas, which produced a grain yield of 1980.7 kg ha^{-1}.

In 2007, plots receiving no N and the plots receiving 112 and 224 kg ha^{-1} N were not statistically different when winter cover-crop peas were present. However, the plots receiving no N, when winter cover-crop peas were present, almost doubled the yield (2546.6 kg ha^{-1}) of the plots receiving no N (1391.2 kg ha^{-1}) when no winter cover crop peas were present. By way of generalized illustration, if N amounts are linearly related to yield, these data imply that the winter-pea cover crop potentially replaced about 80% of the mineral N needs of corn 2007 and about 63% of those needs in 2006.

Fig. 1 shows graphically what was found in 2006 and 2007 when no winter-pea cover crop was present. From the 0 N value to the 112 kg N value, a steep increase of yield is observed. This indicates that corn responded vigorously to the N application when no winter cover-crop peas were present. Fig. 2 shows the same comparison as 2006 and 2007 but with the winter-pea cover crop. From the 0 N value to the 224 kg N value, the line remains relatively flat. This linear expression indicates that the corn did not respond to the N application when winter cover-crop peas were present because all, or a great percentage, of the corn's N need was being met by the pea cover crop.

The relationship between the companion-crop cowpea seeding rate and corn yields is shown in Fig. 3. In 2006 and 2007 when companion-crop cowpeas were included, the corn yields decreased dramatically. In 2006, there was a decline as companion-crop seeding rates increased but it was not significant. However, in 2007 the decline was significant at the $p=0.05$ level of probability and the LSD value was 148. Since the difference between "0" at 3050 and "13" at 2855 was 195 kg ha^{-1} (greater than the LSD), it was significantly different. These results conclude that the companion-crop cowpeas acted as a "weed" within the corn crop by essentially competing very effectively with the corn for water and nutrients.

Pea dry-matter production, nitrogen content of the dry matter, and total nitrogen produced are shown in Table 2. Tissue analysis of the dry peas showed nitrogen content (at roughly 3%) was very consistent over the two years. Dry matter and total N produced over the two years, however, varied somewhat. This may have contributed to the statistically significant yield data (Table 1) found in 2007 when analyzing plots receiving 0, 112, and 224 kg ha^{-1} N where winter cover-crop peas were present. Total nitrogen produced and mineralized in 2007 was roughly 15 kg ha^{-1} less than 2006 values and may have contributed to lower yield where no nitrogen was applied. Also, pea biomass produced in 2007 was 278 kg ha^{-1} less than what was produced in 2006. The plants were exposed to a killing frost in on March 28, 2007, which could have contributed to the reduction of pea biomass during the 2007 growing season. Additionally, moisture levels received between January 1 and April 30, 2007 were approximately 31% lower than for the same period in 2006 (Table 3). Perhaps a combination of the hard freeze and 31% decrease in spring rainfall reduced pea biomass and therefore reduced N that could be fixed by plants and mineralized in the soil for use by the corn.

Table 3 shows the effect the cover crop had on soil moisture. In both years, the cover crop used about twice as much soil moisture, as measured in the upper 0-6 cm

of soil, as did the side of the field without winter-pea cover crop. The rainfall in both years was normal, but 31% less in 2007. In any case, no decline in corn germination was seen in either year. However, both spring 2006 and 2007 had normal rainfall levels. In a dry spring, soil moisture in the winter-pea cover-crop area might be low enough to affect corn germination. In excessively wet years, it is possible that moisture taken up by the winter peas may allow cultivation or planting to take place earlier due to promotion of soil-drying conditions.

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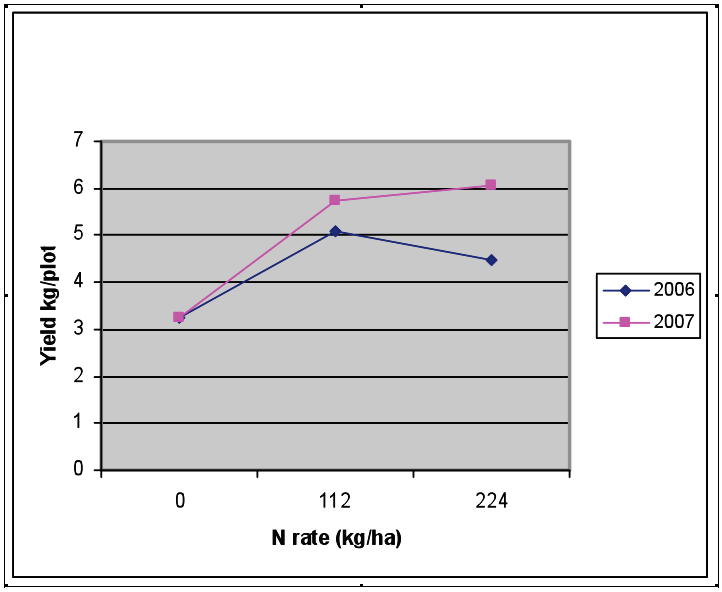

Fig 1. Corn yield when no winter-pea cover crop was present

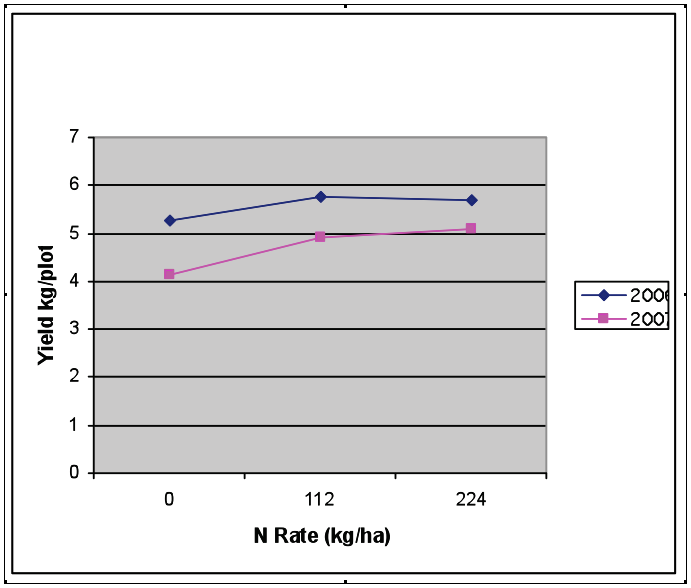

Fig 2. Corn yield when winter-pea cover crop was present

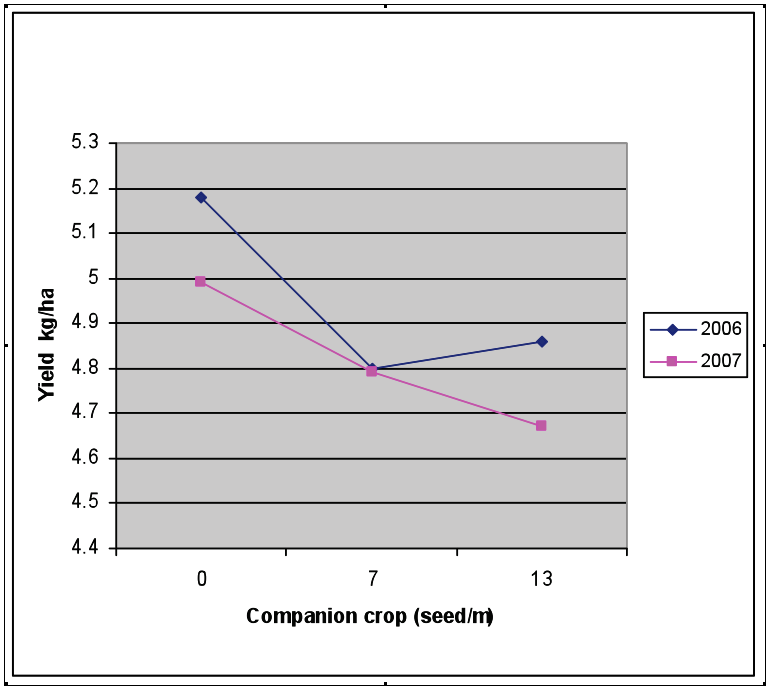

Fig 3. Effect of companion-crop seeding rate on corn yield
LSD (148.5) is used for comparison of treatment means in 2007, and was significant at the p=0.05 level of probability.

Table 1. Corn yields with varying combinations of fertilizer and companion crop strategies. 2006 & 2007 plot yield values from Fayetteville, Ark.

Year	Level of nitrogen(kg/ha^{-1})	Yield values (kg/ha^{-1})	
		Winter-pea cover crop	No winter-pea cover crop
2006	0	3230.4	1980.7
	112	3537.0	3136.1
	224	3489.8	2617.4
2007	0	2546.6	1391.2
	112	3018.2	3537.0
	224	3112.6	3725.6

LSD (725.9) for comparison of means of different years at the same combination of winter peas and nitrogen treatments
LSD (750.8) for comparison means of different nitrogen treatments at same winter-pea treatment
LSD (1,213.5) for comparison of means from different levels of winter-pea treatment

Table 2. Winter pea dry matter production and plant tissue nitrogen content for 2006, 2007

Year	Dry matter (kg/ha^{-1})	N (%)	Total N (kg/ha^{-1})
2006	1210.77	3.07	44.81
2007	932.66	3.11	29.00

Table 3. Soil moisture levels at corn planting, and total rainfall for the previous 4 months

Year	*Winter peas present (m^2/m^2)	Winter peas absent (m^2/m^2)	Rainfall, previous 4 month. (cm)
2006	0.137	0.269	34
2007	0.202	0.388	23

*moisture samples taken just before winter peas were cut and incorporated into soil.

Effects of storage temperature and duration on the milling properties of rice

Tanya Pereira[*]*, Nora Cooper*[†]*, and Terry Siebenmorgen*[§]

ABSTRACT

To maximize rice quality, it is essential to quantify the various factors that affect milling properties of rice. Rice aging, a process during which rice undergoes a series of chemical and physicochemical changes, affects head rice yield (HRY) and the rate at which HRY changes with degree of milling (DOM). This study examined effects of storage duration (0, 2, and 4 months) and storage temperature (4, 21, and 35°C) on milling properties of 'Wells' (long-grain) and 'Jupiter' (medium-grain) rice cultivars. In general, HRY increased with storage duration, most significantly for Wells cultivar. Millability curves were developed by plotting HRY vs. surface lipid content (SLC) of milled rice. Millability curves of Wells had greater slopes, 11.3 pp decrease in HRY for every 1.0 pp decrease in SLC, than those of Jupiter, 8.5 pp decrease in HRY per 1.0 pp decrease in SLC.

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MEET THE STUDENT-AUTHOR

Tanya Pereira

I graduated from Indian School Muscat in Oman in 2004 and enrolled at the University of Arkansas in the fall as a biological engineering major. Since my freshman year at the university, I have been involved with on-campus organizations. I have been a Resident Assistant on campus since 2006 and have served on the International Students Organization committee for two years. I enjoy participating in community service activities and participated in Alternative Spring Break as a Hurricane Katrina relief work volunteer in 2006 and 2007. I have also participated in several leadership and diversity programs including the Emerging Leader Program, Resident Interhall Congress, and the Diversity Leadership Program. During my junior year, I began working for Dr. Siebenmorgen who got me interested in the rice processing research area. Upon graduating, I plan to attend graduate school and pursue a career in the food processing industry. I would like to give a very special thanks to my research mentor, Nora Cooper, for her support and guidance throughout this project and to my faculty mentor, Dr. Siebenmorgen, for giving me the opportunity to be a part of this exciting project.

INTRODUCTION

Rice, the most widely consumed cereal grain in the world, is typically eaten as a milled, intact kernel. Consumers prefer rice to be white and polished, and thus rough rice is processed to first remove the hull and then milled to remove the germ and bran layers. Head rice yield is the current standard used to measure rice milling quality and is defined as the mass percentage of rough rice that remains as head rice (kernels that are at least three-fourths of the original kernel length) after complete milling (USDA, 2005). The milling rate determines the duration required to achieve a given DOM, or the extent to which the bran layers of brown rice kernels are removed during the milling process. Degree of milling can be rapidly determined by light transmittance or by near infrared (NIR) spectroscopy techniques. Rice bran and germ comprise 15-20% lipids (Juliano, 1985) and thus lipid extraction methods, which are presumably more accurate than rapid methods, can be used to measure SLC of milled rice kernels as a measure of DOM. As rice is milled to greater extents, a greater DOM is achieved, leading to decreased SLC; HRY decreases linearly with decreased SLC (Reid et al., 1998).

Since DOM affects profit for rice farmers and millers in terms of HRY reduction, it is essential to understand the relationship between the various factors that affect milling properties of rice, one of which is aging. Rice aging is a complicated process during which rice undergoes a series of chemical and physicochemical changes caused by interactions between kernel components such as starch, lipids, and proteins. These interactions affect kernel hardness, which influences the rate at which bran is removed during milling (Bhashyam and Srinivas, 1984; Pomeranz and Webb, 1985). Studies on aging of rice have shown that tensile strength, hardness, and resistance to grinding increased after storage (Kondo and Okamura, 1937, Kunze and Choudhury, 1972). Daniels et al. (1998) found that rice required approximately 50% longer milling durations to reach the same DOM as achieved prior to aging, indicating that longer storage durations may yield harder kernels, thus making it more difficult to remove bran during the milling process. The above-mentioned changes occur most rapidly during the first few months of storage at a temperature of 15°C (Perez and Juliano, 1982). Head rice yield increases with storage duration (Daniels et al., 1998; Villareal et al., 1976), however, the effects of storage duration and storage temperature on the rate at which HRY changes with SLC, or the millability of rice, have not yet been quantified. Therefore, the objective of this study was to examine effects of storage temperature and duration on the millability of rice.

MATERIALS AND METHODS

Sample procurement and storage conditions. In fall 2007, rice cultivars Wells (long-grain, 16.6% harvest moisture content (MC)) and Jupiter (medium-grain, 16.7% harvest MC) were harvested from Stuttgart, Ark., and Newport, Ark., respectively. Samples were cleaned (Kicker Grain Tester, MidContinent Industries, Inc., Newton, Kan.) and then dried in a chamber maintained at 26°C and 56% relative humidity, corresponding to a rough rice equilibrium MC of 11.5% (ASAE 2004). When the rough rice MC had reached 12.5–12.9%, determined as the average MC of 50 kernels measured with an individual kernel MC meter (CTR 800E, Shizuoka Seiki, Shizuoka, Japan), each cultivar lot was separated into 56 samples of 150 g each, which were sealed in Ziploc bags. Eight of the samples represented replications 1 and 2 (four samples each) for the month-zero storage duration and were subjected to milling tests without storage duration or storage temperature treatment. The remaining 48 samples of each cultivar were grouped into six lots of eight samples each. The six lots were allocated to chambers maintained at 4, 21 or 35°C with two lots, representing replicates one and two, being allocated to each chamber. After two and four months, four samples were removed from each replication at each storage temperature and subjected to milling tests.

Milling procedure and head rice yield determination. Prior to milling, rough rice samples were removed from storage and, while still in the sealed plastic bags, allowed to equilibrate to room temperature for at least one day. Four samples from each cultivar/storage temperature/storage duration/replication combination were dehulled in a laboratory huller (Rice Machine, Satake Engineering Co., Hiroshima, Japan) with a clearance of 0.048 cm (0.019 in) between the rollers as specified by USDA (1982). The resultant brown rice samples were milled using a laboratory mill (McGill No. 2, RAPSCO, Brookshire, Texas) equipped with a timer. A 1,500-g mass was placed on the mill lever arm 15 cm from the center of the milling chamber. The four samples of each replication were milled for either 10, 20, 30, or 40 s in order to create samples of varying DOM, and then aspirated (Grain Blower, Seedburo Equipment Co, Chicago, Ill.) for 30 s to remove excess bran particles. Head rice was then separated from brokens using a sizing device (Seedburo Equipment Co., Chicago, Ill.) and HRY was expressed as the mass percentage of head rice to initial rough rice mass. Milled rice samples were stored at 4°C until SLC measurement.

Surface lipid content measurement. Head rice samples were removed from cold storage and allowed to equilibrate to room temperature for 1 h prior to SLC determination. The SLC of each head rice sample was measured, in duplicate, using a lipid extraction system (Soxtec Avanti 2055, Foss North America, Eden Prairie, Minn.) following the procedure developed by Matsler and Siebenmorgen (2005). This method used 5 g of head rice weighed into cellulose thimbles (Foss North America, Eden Prairie, Minn.). The thimbles and head rice were first pre-dried for 1 h in an oven maintained at 100°C. Lipid was then extracted from the sample by immersing the thimbles in extraction cups containing boiling 70 mL of petroleum ether (boiling point 35–60°C; VWR, Suwanee, Ga.) for 20 min. The thimbles were then raised above the solvent and the samples rinsed with petroleum ether condensate for 30 min. After rinsing, the extraction cups were placed into an oven maintained at 100°C for 30 min to evaporate any solvent in the cup, then moved to a desiccator where the cups were cooled to room temperature for 30 min before being weighed. The difference between the mass of the cups containing the extracted lipid and the original mass of the cups was then calculated to obtain the mass of the extracted lipid. Surface lipid content was expressed as the mass percentage of extracted lipid to the original head rice sample mass. Duplicate measurements were averaged before data analysis.

Statistical analysis. Using statistical software (JMP 7, SAS Institute, Inc., Cary, N.C.), the means obtained from the HRY and SLC tests were analyzed using least squares regression with milling duration, storage duration, and storage temperature as independent variables. Significance was determined using a student's t-test with an alpha level of 0.05. To compare the effects of HRY vs SLC, a full factorial model was constructed in a least squares regression analysis to determine effects of the independent variables (storage temperature, storage duration, and SLC) on HRY. Regression lines relating SLC and HRY were each constructed using eight data points (four milling durations, two replications).

RESULTS AND DISCUSSION

Figures 1 and 2 show effects of storage temperature and duration on the SLC of Wells and Jupiter rice cultivars, respectively, milled for 10, 20, 30 and 40 s. Surface lipid content decreased with increased milling duration, as anticipated from studies such as Cooper and Siebenmorgen (2007) and Siebenmorgen et al. (2006). After two and four months of storage, SLCs of both Wells and Jupiter (Fig. 1 and Fig. 2) did not vary significantly from those at zero months of storage except at the highest storage temperature of 35°C after 4 months. Storage temperature significantly affected SLCs of Jupiter, but storage duration did not. Both storage dura-

tion and temperature significantly affected the SLCs of Wells without an interaction between these independent variables.

Figures 3 and 4 show effects of storage temperature and duration on HRYs of Wells and Jupiter, respectively, milled for 10, 20, 30, and 40 s. Head rice yields decreased with increased milling duration. After four months of storage, (Fig. 3b), HRYs of Wells stored at 35°C were significantly greater than those stored at 4 and 21°C, as well as those with no storage. For this cultivar, storage duration significantly affected HRY, as did interactions between storage temperature and milling duration, and between storage temperature, storage duration, and milling duration. After two months of storage (Fig. 4a), there was no significant difference between HRYs of Jupiter stored at 4, 21 and 35°C and HRYs at zero months of storage. After four months of storage (Fig.4b), HRYs of Jupiter stored at 21°C were significantly greater than at zero months of storage and were not significantly different than Jupiter stored at 35°C. Storage duration significantly affected the HRY of Jupiter; there was also a significant interaction between storage temperature and storage duration.

Millability curves relating HRY to SLC are shown in Figures 5 and 6, which were developed by plotting HRYs vs. the corresponding SLCs of Wells and Jupiter cultivars, respectively, milled for 10, 20, 30, and 40 s after having been stored at 4, 21 and 35°C for 0, 2 and 4 months of storage. Both figures show that HRYs were linearly related to SLC; HRY decreased as SLC decreased. This trend was previously noted by Cooper and Siebenmorgen (2007) and Reid et al. (1998). Slopes of the regression lines of each storage duration/storage temperature combination can be found in Table 1. These slopes indicate the change in HRY that can be expected with a unit percentage point (pp) change in SLC. For example, the HRY of Wells at 0 months of storage changed 12.1 pp for every 1.0 pp change in SLC. Even though there was a distinct upward shift in HRYs after four months of storage (Fig. 5a), the rate of change in HRY with SLC only varied from 10.6 to 11.5 pp per 1.0 pp change in SLC, except for at two months of storage and 4°C storage temperature, when the slope of the regression line was 15.9 (Table 1). The millability slope for this storage temperature/duration combination was significantly different than the other slopes. The average of all Wells slopes was 12.0, though it is suspected that the 15.9 slope for month 2/4°C was an anomaly; if omitted, the average slope was 11.3.

Millability regression line slopes of Jupiter were of a lesser magnitude than those of Wells (Fig. 6, Table 1), which could be attributed to the geometry differences of the long-grain vs medium-grain cultivars. At zero months of storage, HRY changed at a rate of 8.3 pp for every 1.0 pp change in SLC (Table 1). The slopes of all Jupiter storage duration/temperature combinations were not statistically different, however, trends were such that the slope decreased to 7.7 after two months of storage at 4°C, and gradually increased with storage duration and storage temperature until reaching 9.2 after four months of storage at 35°C. On average, the HRY of Jupiter changed 8.5 pp with a 1.0 pp change in SLC.

The average slope of all millability regression lines produced in the current study was 9.9 (Table 1), which is only slightly greater than the average slope of 9.4 found by Cooper and Siebenmorgen (2007). Cooper and Siebenmorgen (2007) included 17 rice samples, including both long- and medium-grain samples, milled for 4 durations and aged for 0, 1, 2, 3 and 6 months. It is possible that further storage may produce samples that mill at a rate similar to that found by Cooper and Siebenmorgen (2007).

Summary. For Wells, storage duration resulted in a significant increase in HRYs after two months of storage at 35°C and after four months of storage at all temperatures relative to the month-zero HRYs. This effect was cultivar-specific as HRY changes due to storage duration or temperature were not observed in the medium-grain cultivar Jupiter. The rate of change in HRY with a change in SLC (millability) was greater in the long-grain cultivar, with an average rate of change of 11.3, than in the medium-grain cultivar, which produced an average rate of change of 8.5. Millability did not change significantly with storage duration or storage temperature, though ongoing research involving longer storage durations could elucidate possible effects of storage conditions on rice millability.

ACKNOWLEDGMENTS

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Table 1. Slopes and coefficients of determination (R^2) of the millability linear regression lines of Figs. 5 and 6, relating the head rice yields and surface lipid contents of Wells and Jupiter cultivars stored for 0, 2, and 4 months at 4, 21, and 35°C.

Cultivar	Storage duration (mos)	Storage temperature (°C)	Slope	R^2
Wells	0	NA	12.1	0.98
	2	4	15.9	0.95
		21	10.6	0.96
		35	11.0	0.94
	4	4	11.2	0.91
		21	11.5	0.95
		35	11.4	0.98
Jupiter	0	NA	8.3	0.98
	2	4	7.7	0.94
		21	8.1	0.98
		35	8.4	0.98
	4	4	8.4	0.89
		21	9.1	0.99
		35	9.2	0.99

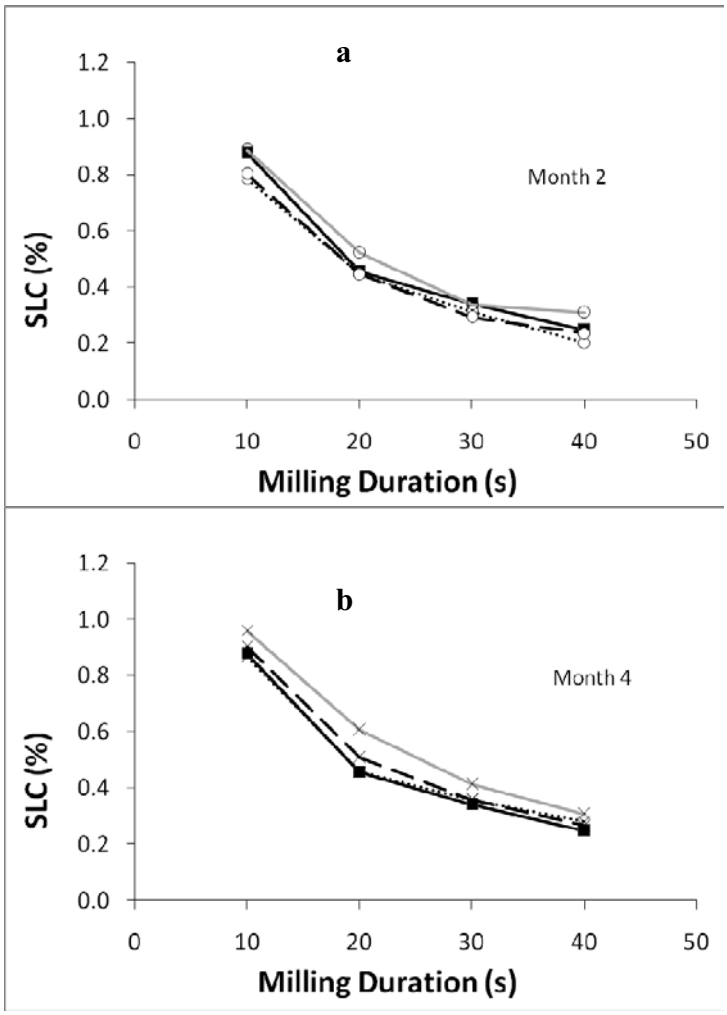

Fig.1. Surface lipid contents (SLCs) of Wells (long-grain) rice cultivar milled for 10, 20, 30, and 40 s after having been stored at 4°C (·····), 21°C (- - - -) and 35°C (——) for a: 0 (■) and 2 (○) months of storage and at b: 0 (■) and 4 (x) months of storage.

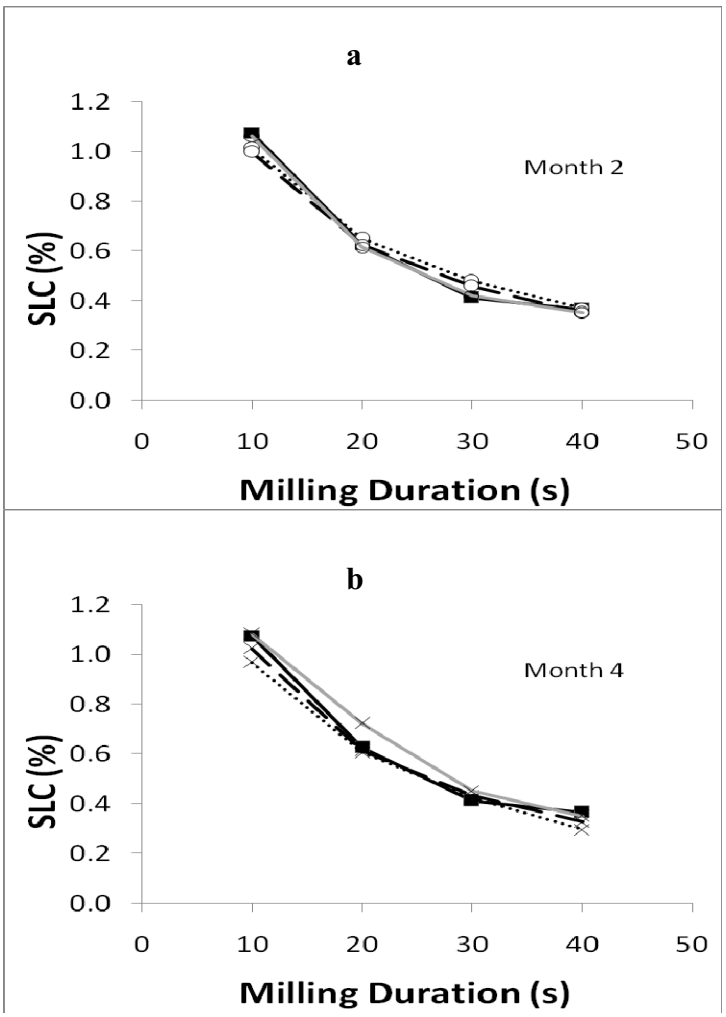

Fig. 2. Surface lipid contents (SLCs) of Jupiter (medium-grain) rice cultivar milled for 10, 20, 30, and 40 s after having been stored at 4°C (·····), 21°C (- - - -) and 35°C (——) for a: 0 (■) and 2 (○) months of storage and at b: 0 (■) and 4 (x) months of storage.

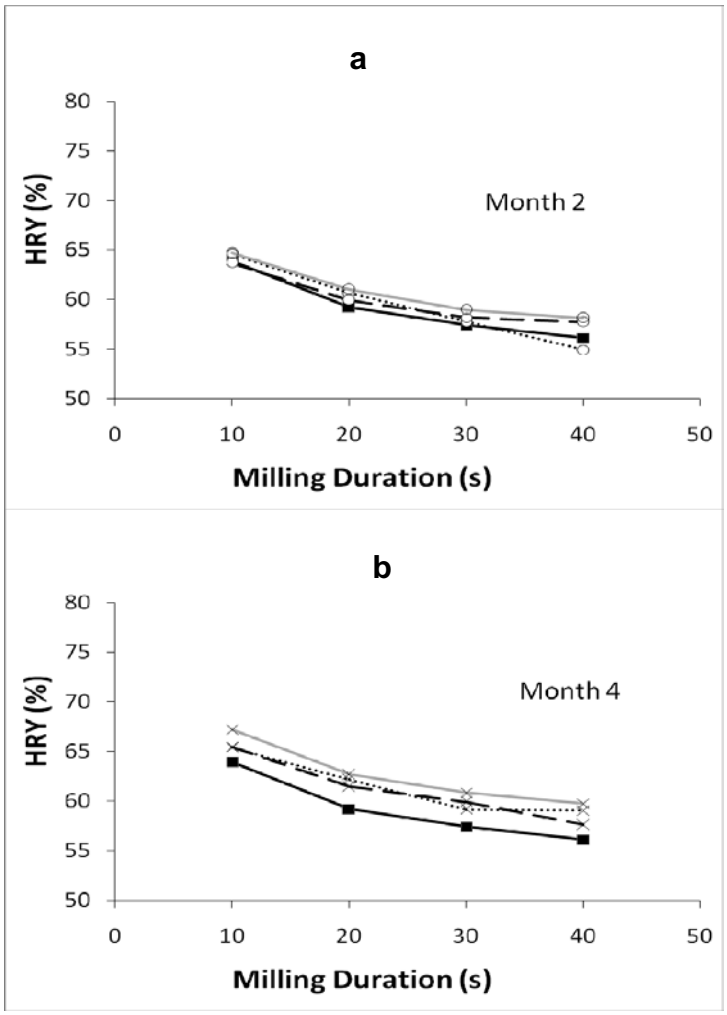

Fig. 3. Head rice yields (HRYs) of Wells (long-grain) rice cultivar milled for 10, 20, 30, and 40 s after having been stored at 4°C (·····), 21°C (- - - -) and 35°C (——) for a: 0 (■) and 2 (○) months of storage and at b: 0 (■) and 4 (x) months of storage.

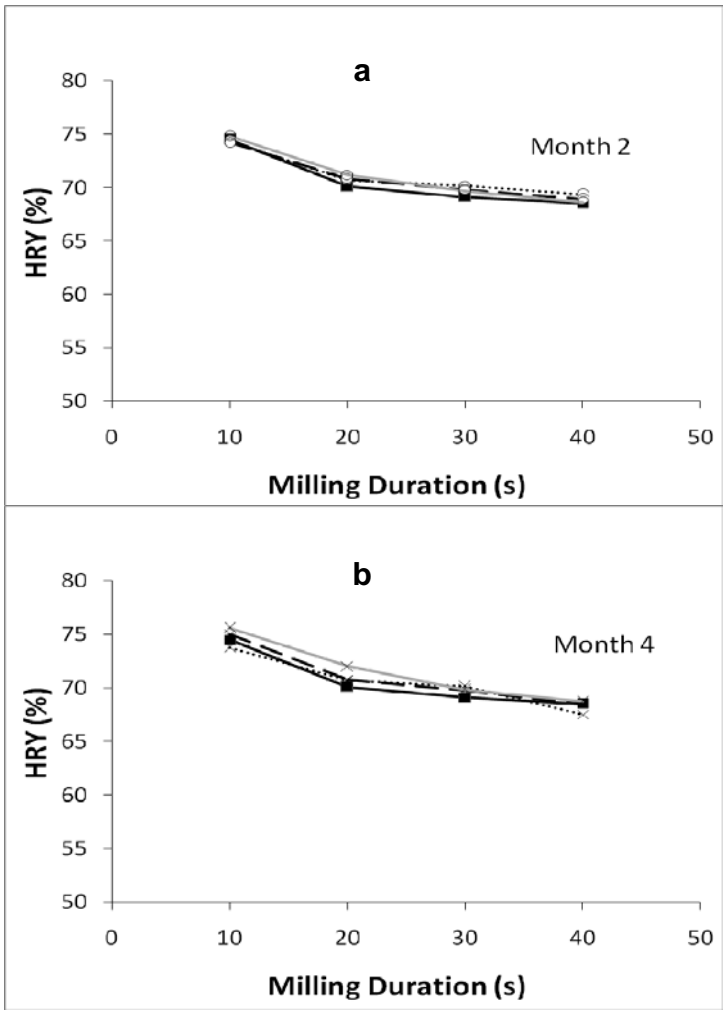

Fig. 4. Head rice yields (HRYs) of Jupiter (medium-grain) rice cultivar milled for 10, 20, 30, and 40 s after having been stored at 4°C (·····), 21°C (- - - -) and 35°C (——) for a: 0 (■) and 2 (○) months of storage and at b: 0 (■) and 4 (x) months of storage.

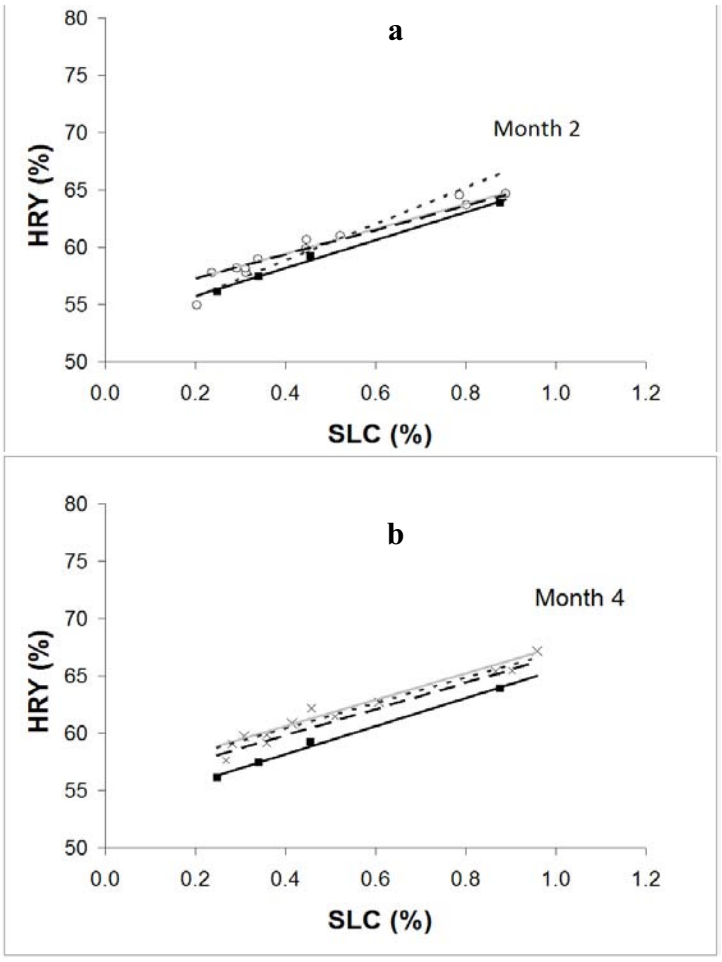

Fig. 5. Head rice yields (HRYs) and corresponding surface lipid contents of Wells (long-grain) rice cultivar milled for 10, 20, 30, and 40 s after having been stored at 4°C (·····), 21°C (- - - -) and 35°C (——) for a: 0 (■) and 2 (○) months of storage and at b: 0 (■) and 4 (x) months of storage.

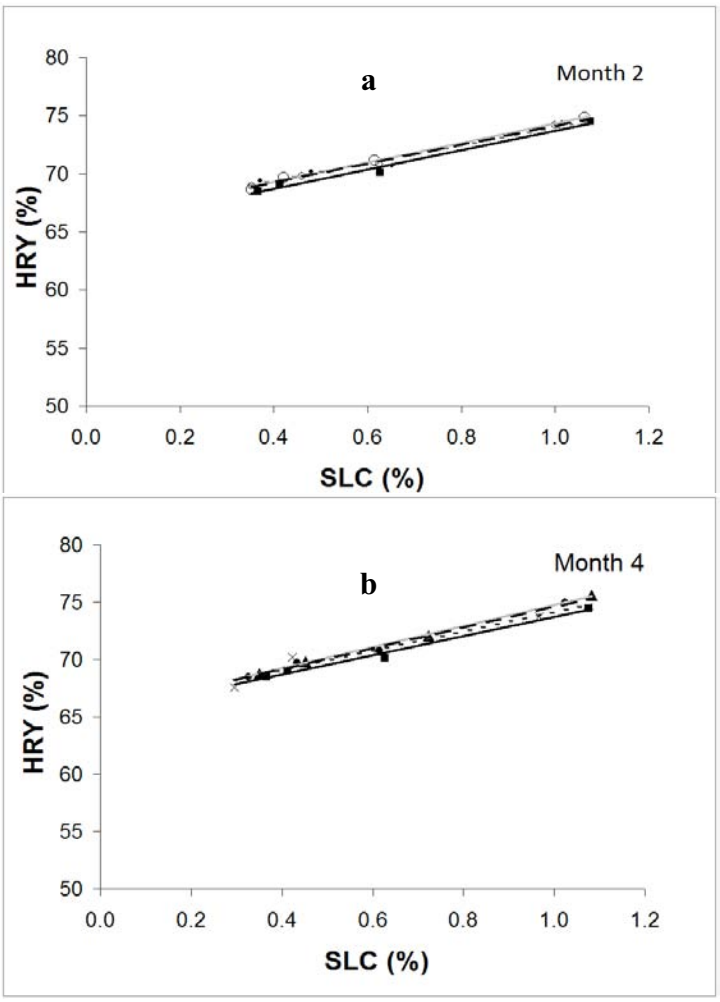

Fig. 6. Head rice yields (HRYs) and corresponding surface lipid contents of Jupiter (medium-grain) rice cultivar milled for 10, 20, 30, and 40 s after having been stored at 4°C (·····), 21°C (- - - -) and 35°C (——) for a: 0 (■) and 2 (○) months of storage and at b: 0 (■) and 4 (x) months of storage.

Earthworm abundances in endophyte-infected tall fescue pastures in Northwest Arkansas

Ashley C. Rashé [*] *and Mary C. Savin*[†]

ABSTRACT

The ecology of organisms that co-evolve within an ecosystem is likely to be distinct from that involving organisms recently introduced into an area. To better understand the relationship of earthworms with endophyte-infected tall fescue, earthworms in novel and toxic endophyte-infected tall fescue pastures were enumerated and identified as adults or juveniles. We hypothesized that differences in endophyte infection of the fescue would influence earthworm abundances. Earthworms in two toxic and two novel endophyte-infected tall fescue fields in Fayetteville, Ark., were sampled weekly from January through July 2007. Each type of endophyte-infected pasture was established in 1997 and 2003. Sampling was carried out utilizing a physical dig-and-sort extraction method. Although variable, sampling time was a significant factor in the number of adult and juvenile worms collected. Adult earthworm abundances showed a seasonal trend of declining numbers from winter to summer, while juvenile worms showed an increase from winter to summer. Previous studies have shown that endophyte infection of plants can impact soil organisms. In this study, type of fungal endophyte infection did not appear to impact earthworm abundances; therefore, use of novel endophyte-infected fescue in a pasture is not expected to have an impact on the ecology of earthworms.

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Ashley Rashé

MEET THE STUDENT-AUTHOR

I graduated from Cassville High School, Mo., in 2004 and enrolled in the University of Arkansas as an honors student in the fall, choosing Environmental, Soil, and Water Science as my major. Shortly thereafter, I also enrolled in the Crop, Soil, and Environmental Sciences Department's new minor, Wildlife Habitat. I am currently a senior and have been actively involved in many campus clubs such as the Crop, Soil, and Environmental Sciences undergraduate club, the Sierra Student Club, and Alpha Zeta Agricultural Fraternity.

My first year of college, I began working in the Soil Biology and Microbial Ecology Laboratory under the supervision of Dr. Mary Savin. I began conducting research about earthworm abundances and distributions in endophyte-infected tall fescue pastures. This became the research project for my thesis, and this experience so early in my college career was especially beneficial. In 2005, I participated in the Undergraduate Club Poster Contest at the American Society of Agronomy (ASA) annual meeting in Salt Lake City. In 2006 and 2007, I presented my research at the ASA conferences in Indianapolis and New Orleans, respectively.

I have received many grants, scholarships, and honors throughout my four-year education. Some of the grants and scholarships I have received are the Dale Bumpers College Undergraduate Research Grant, Honors College Grant, the Fontaine R. Earl Scholarship, and the Arkansas State Plant Board Intern Scholarship. I was awarded the National Student Recognition Award by ASA in 2007.

The opportunities I have experienced with the Department of Crop, Soil, and Environmental Sciences have allowed me to succeed in receiving a solid education and gain knowledge for my future endeavors. I could not have asked for better professors and classmates than those I had at the Dale Bumpers College of Agricultural, Food and Life Sciences.

INTRODUCTION

Earthworms are keystone species that can substantially impact biological, chemical, and physical properties of terrestrial ecosystems. Epigeic worms reside in the litter layer and surface soil and consume litter. Anecic earthworms pull plant litter from the ground surface and incorporate it into the soil in burrows. Endogeic earthworms live within the soil horizons, constructing both horizontal and vertical burrows.

Earthworms can recycle nutrients by moving litter into soil, facilitating decomposition, and making nutrients plant available (Amador et al., 2005), thereby increasing nitrogen (N) concentration in vegetative tissue (Callaham and Hendrix, 1998). Earthworms also impact ecosystems through physical changes such as altering aggregates and macropore formation, consequently increasing aeration and allowing easier drainage and less runoff. Anecic earthworm burrows have been found to facilitate faster movement of water and chemicals applied directly to the soil surface through the soil matrix (Shipitalo et al., 1999). However, anecic earthworm burrows can also have detrimental effects. Chemicals applied frequently and in large amounts can be transported rapidly in macropores, and are thus not attenuated during filtration through the soil matrix (Shipitalo and Butt, 1999).

Any impact on earthworm ecology is important to ecosystem functioning. Earthworms are generally considered keystone species and beneficial in agricultural systems because they are ecosystem engineers and have a disproportionately large effect on ecosystem functions, such as decomposition and nutrient cycling. Tall fescue (*Festuca arundinacea* Schreb) is a cool-season grass used commonly in pastures in the United States, particularly in humid regions such as the Southeast (Franzluebbers

and Stuedemann, 2005). It is infected with a fungal endophyte that helps the plant but is toxic to cattle during times of the year of high abiotic stresses, such as drought or disease prevalence (Humphries et al., 2000). Inputs of alkaloids from the endophyte into the soil have been shown to alter soil carbon (C) structure through reduction of microbial activity (Franzluebbers and Hill, 2005). Other organisms such as earthworms that consume plant litter may also be affected by the toxin production. In addition to affecting the resource quality of litter, the type of endophyte in symbiosis with the fescue could impact earthworms negatively by altering utilization of belowground resources and nutrients by the plant. In turn, this can affect the relationships that plants have with other soil organisms (Omacini et al., 2005).

Geographical distribution of different earthworm populations has important implications for the effects of worms on an ecosystem. For example, non-native (or exotic) earthworms have profoundly altered physical and biogeochemical properties of northern forests. The forests were previously uninhabited by earthworms. Introduction of worms altered carbon and nitrogen pools, as well as caused a complete loss of the forest floor horizon due to increased decomposition. Although decomposition occurs mainly through activity of microorganisms, earthworms drastically altered root distribution and functioning, reduced pools of C and N through hydrologic and gaseous losses and P through hydrologic losses, and affected the activity of the microbial community (Bohlen et al., 2004).

Arkansas is an example of a state where both native and exotic earthworms overlap in distribution; however, earthworm populations in Arkansas are largely unknown. Population identifications have not been published since the 1950's (Causey, 1952 and 1953) and this information is needed for future ecological studies. We set out to enumerate earthworm abundances in one type of managed ecosystem so that we could also later identify species and investigate population distributions.

The objectives of this study were to determine if earthworm abundances were different under novel and toxic endophyte-infected tall fescue pastures, and how abundances in each of those pastures changed seasonally from January to July. Given the potential for changes in litter quality from the presence of toxic versus non-toxic endophyte, endophyte infection of tall fescue was hypothesized to affect earthworm abundances.

MATERIALS AND METHODS

Study site. The study site consisted of four 1.62-hectare pastures growing tall fescue (*Festuca arundinacea* Schreb). The sites were located in Fayetteville, Ark., a northwest region of the state. Two fields were growing novel endophyte-infected fescue (*Neotyphodium coenophialum* Glenn, Bacon & Hanlin) and two were growing toxic endophyte-infected (*Neotyphodium coenophialum*) tall fescue. One pasture of each endophyte-infection type was planted in 1997 and one pasture of each endophyte-infection type was planted in 2003. All sites had minor amounts of crabgrass, yellow foxtail, and bermudagrass.

Worm collection. Worms were generally collected twice a week from January through July 2007. For another portion of this study not being reported here, we had performed a comparison of two different earthworm extraction methods, i.e. chemical expulsion using a mustard solution and a physical dig and sort method. Worm abundances presented here are from the dig and sort method only. From January to the end of May, dig and sort directly followed use of mustard extraction on the same area, so numbers under-represent total abundances during that time period. However, mustard extraction was variable and not very efficient, so abundances are presented as collected following the application of a mustard solution poured onto a 30 x 30 cm^2 area. Worms were collected for 20 to 40 min (Chan and Munro, 2001). The area was then dug to a depth of 20 cm and soil was removed, spread on a tarp, and sifted through by hand to find earthworms. From the end of May to July, only the dig and sort method was used to collect worms. On each sampling date, one to all four pastures were sampled. In each pasture sampled, three plots (30 x 30 cm^2) were sampled along a transect.

In the field, worm abundances were recorded and worms were stored in specimen cups lined with moist paper towels. Upon return to the laboratory, worms were dipped in boiling tap water to kill them quickly. They were then placed in test tubes with 5% formalin for preservation. Boiling minimizes constriction of the earthworms segments. Earthworms were counted and identified by external features as adults or juveniles. Some worms were not intact after collection and preservation. Unless there was enough of the worm to distinguish adult or juvenile, the worm was not identified as either stage.

Data analysis. Averages (per 30 cm^2 or 0.09 m^2 area) were calculated for total earthworm abundances and for numbers of adults and juveniles, and for each endophyte-infection type for each date sampled, regardless of year that the pasture was planted. For each of the datasets (total, adult, or juvenile abundances) from the dig and sort method, two arbitrary linear regression lines were fit simultaneously: one for toxic endophyte and one for novel endophyte-infected fescue. The fitted lines were tested for equality of slopes. We concluded

that the slopes were equal, i.e. with a *P* value greater than 0.05, and therefore we fit a new model consisting of two parallel lines, one for toxic and one for novel endophyte infection. The fitted parallel lines were tested for equal intercepts. If we concluded that the intercepts were equal, then we fit one line to data combining both types of endophyte infection. No adults were found in late June and July, so the values of zero were removed from the dataset used in the regression analysis of adult numbers through time because those data have no variability.

RESULTS AND DISCUSSION

Average earthworm abundances ranged from 0 to 12 earthworms (per 0.09 m^2 area sampled) throughout the study period (Fig. 1). The slopes ($P = 0.69$) and y-intercepts ($P = 0.71$) of earthworm abundances in soil growing toxic versus novel endophyte-infected tall fescue were not different, causing the regression lines to collapse into one (Fig. 1). Statistically, total earthworm abundances in these pastures were not impacted by endophyte infection.

Temporally, total abundances showed a slight, but not significant, linear decrease over time ($P = 0.055$). Results of other studies have shown that sampling during a similar time period, winter through early summer, was most effective for collecting the highest number of worms. For example, Callaham and Hendrix (1997) found the highest earthworm abundances in a forest ecosystem in Georgia in late spring and early summer and lowest abundances in late summer and autumn. Earthworms numbered 75-80 earthworms per m^2 or approximately 7 earthworms per 0.09 m^2. Although abundances were variable among collections in this and other studies, and comparisons of worm numbers among studies, or across years, may be confounded by fluctuations of soil temperature and moisture, abundances were similar to the findings of our study.

Adult earthworm numbers ranged from an average of 0 to 11.3 earthworms (per 0.09 m^2 area sampled) (Fig. 2.) The slopes ($P = 0.97$) and y-intercepts ($P = 0.87$) were the same for both types of endophyte infection and so were collapsed into one line (Fig. 2). The regression line showed a significant linear decrease over time and adult worms were not present in late June and July ($P < 0.0001$, Fig. 2).

Juvenile earthworm abundances ranged from an average of 0 to 10.3 earthworms (per 0.09 m^2 area sampled) (Fig. 3.) Again, the slope ($P = 0.45$) and y-intercept ($P = 0.54$) were the same for both types of endophyte infection, and so were collapsed into one line. In contrast to the trend in adult numbers, the collapsed regression line showed a significantly linear increase over time ($P=0.0064$, Fig. 3). Therefore, while endophyte infection did not have an impact on either adult or juvenile worm abundances, we did observe apparent time trends in adult and juvenile abundances. The slope for the adult abundances was steeper than that found for juveniles because adult numbers declined to zero in late June, but juveniles were found throughout the study period.

Our data showed that juvenile abundances significantly increased winter through summer. In a study conducted in Georgia under a forest ecosystem, juvenile earthworms were most abundant November through May, and total abundances were greatest in late spring through early summer (Callaham and Hendrix, 1997). Adults were present, but not in as high abundances as juveniles and, similar to our study, adult abundances slowly began to decrease after May. The decrease in soil moisture in this study (data not shown) may partly explain the absence of adult earthworms in late June and July. This seasonal trend may also imply that once adult earthworms have reproduced, contributions to ecosystem functions will be dependent on juvenile earthworm survival and growth.

For research performed in the tall fescue pastures of Northwest Arkansas, the most appropriate times for sampling to collect highest abundances of worms would be January through late June. However, adult worms are necessary to identify earthworm species. Therefore, sampling for identifications should be conducted in late winter through early spring or January through late May, when adults are present.

Information about the effects that plant endophyte infection status has on soil organisms will allow for better land management decisions (Humphries et al., 2000). The difference in endophyte infection among fescue pastures had no significant impact on earthworm abundances in this study. We had expected that type of endophyte infection (toxic versus novel) would impact earthworm populations based on the ecological importance of earthworms in decomposing plant residues. The lack of significant differences in earthworm abundances, of both juveniles and adults, suggests that whether tall fescue is infected with the toxic or the novel endophyte, it will not impact the ecological relationships with earthworms. Our data suggest that use of fescue that is favorable for cattle, i.e. without the effects of the toxic endophyte, is manageable without compromising the integrity of the ecosystem.

ACKNOWLEDGMENTS

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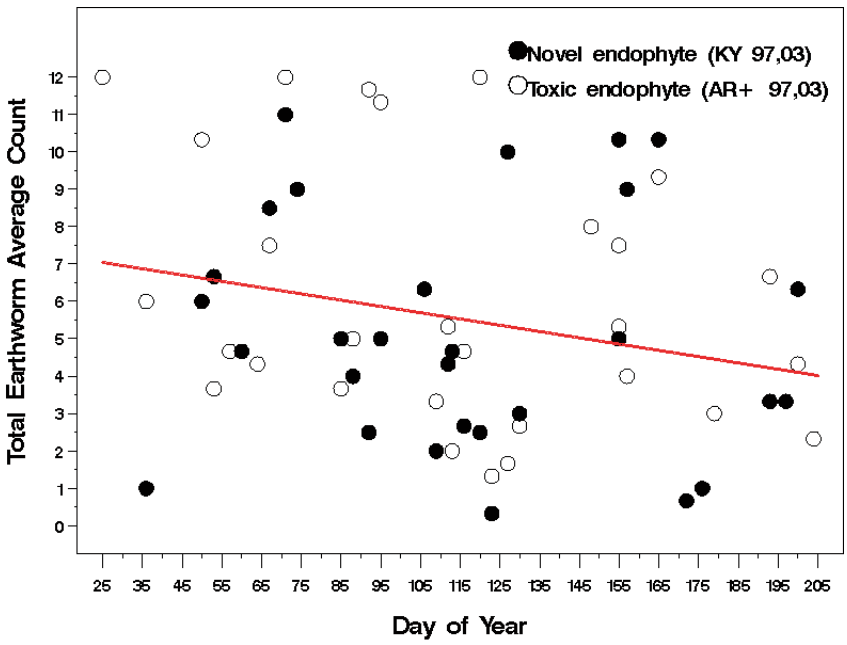

Fig. 1. The average of total earthworm abundances found in novel and toxic endophyte-infected fescue plots from January to July, 2007 (n = 2 - 6). The novel endophyte-infected fescue is indicated as AR+ 97,03 and the toxic endophyte-infected fescue is expressed as KY 97,03.

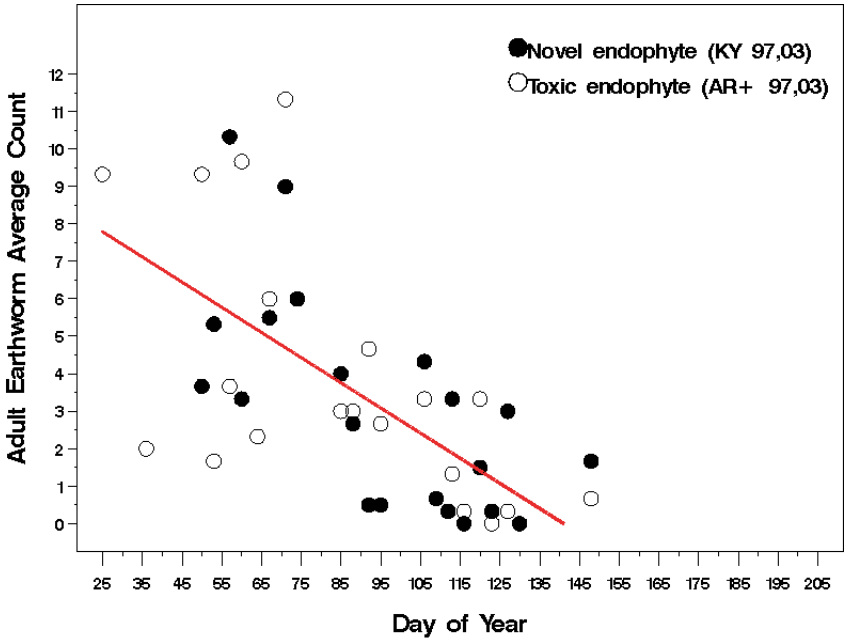

Fig. 2. The average of adult earthworm abundances found in novel and toxic endophyte-infected fescue plots from January to July 2007 (n = 2 - 6). The novel endophyte-infected fescue is indicated as AR+ 97,03 and the toxic endophyte-infected fescue is expressed as KY 97,03.

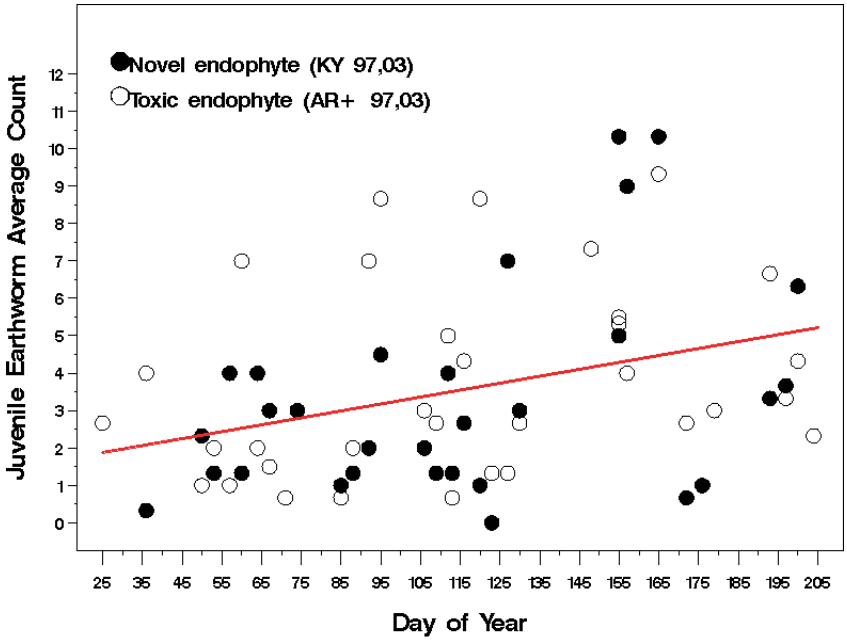

Fig. 3. The average of juvenile earthworm abundances found in novel and toxic endophyte-infected fescue plots from January to July 2007 (n = 2 - 6). The novel endophyte-infected fescue is indicated as AR+ 97,03 and the toxic endophyte-infected fescue is expressed as KY 97,03.

Subcritical water and carbonated water extraction of anthocyanins from grape pomace

Lydia Rice[*] *and L.R. Howard*[†]

ABSTRACT

Grape pomace, a by-product of juice and wine processing, is a rich source of anthocyanins, antioxidant compounds that may afford protection against cancer and coronary heart disease. Unfortunately, traditional extraction of these antioxidants involves use of organic solvents, which pose serious safety and disposal problems for industry. Clearly a need exists for "green" extraction technologies—such as use of subcritical water—that eliminate or reduce the amount of organic solvents. In this study, we determined the efficacy of subcritical and carbonated water in extraction of anthocyanins from red grape pomace. Extraction variables including particle size, pomace mass, and temperature were optimized, and results were compared with those obtained using a traditional solvent-extraction method. According to the total anthocyanin assay, optimum conditions for extraction consisted of the smaller particle size (400 μm) and temperature of 100°C. Under these conditions, subcritical water and carbonated water extracted about 70% of anthocyanins obtained using the traditional organic solvent method. The highest antioxidant-capacity value measured by the ORAC assay was obtained at 140°C, suggesting that Maillard browning products were produced when grape pomace was exposed to increasing temperatures. Subcritical water appears to be a promising, environmentally benign technology to recover health-promoting compounds from grape-processing waste.

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MEET THE STUDENT-AUTHOR

I graduated from Joplin High School in 2004 and enrolled at the University of Arkansas in the fall as a food science and Spanish major. I plan to complete my B.S. in food science and my B.A. in Spanish in May 2008.

During my time here at the University of Arkansas, I have been awarded an Honors College Fellowship, Honors College Undergraduate Research Grant, and two Honors College Study Abroad grants. I have attended the University of Valencia in Valencia, Spain, and the Copenhagen Business School in Copenhagen, Denmark. As a junior, I began working in Dr. Howard's functional foods laboratory because of my interest in nutritional science and antioxidants. Shortly thereafter, I began my own project focusing on the subcritical-water extraction of anthocyanins from grape pomace.

I have thoroughly enjoyed my time at the University of Arkansas Food Science Department, and I plan to pursue my graduate studies here. The University of Arkansas recently awarded me a Distinguished Doctoral Fellowship that will enable me to begin my Ph.D. in fall 2008. I hope to eventually become a food science professor at a university.

Lydia Rice

INTRODUCTION

Dark-pigmented red grapes are a rich source of anthocyanins, antioxidant compounds with an array of health benefits (Bravo 1998; Hou 2003). These antioxidants have been shown to exhibit anticarcinogenic (Bomser 1996), antimutagenic (Gasiorowski et al., 1997), anti-inflammatory, and antioxidative properties (Wang et al., 1999). Moreover, anthocyanins can promote better eyesight (Timberlake et al., 1988), protect against declines in age-related brain function (Joseph et al., 1999), and prevent lipid oxidation that can lead to clogged arteries (Acquaviva et al., 2002; Folts 1998).

When grapes are used for wine-making, only 30% of these beneficial antioxidants are extracted, while a large amount remains in the pomace (Mazza 1995). Therefore, there is much interest in recovering phenolics from pomace waste material for utilization in value-added products such as nutraceutical ingredients or natural food colorants (Barbagallo et al. 2003). Such products would satisfy the growing consumer demand for natural foods that promote general health. Natural anthocyanin-rich extracts isolated from grape pomace are an excellent candidate to fulfill this demand.

Anthocyanins are water-soluble compounds that give fruits, vegetables, and flowers their blue, purple, red, and orange colors. Anthocyanins are flavonoids, a type of phenolic compound that fall within the class of secondary plant metabolites. Phenolics are grouped by the presence of one or more aromatic rings and one or more hydroxyl groups (Cacace and Mazza, 2007). There are six main types of anthocyanins including cyanidin, delphinidin, malvidin, peonidin, petunidin, and pelargonidin. The predominant anthocyanin in wine grapes is malvidin-3-glucoside (Passamonti et al., 2003).

Consumer demand often drives technological advances in industry in that while consumers want antioxidant-rich products, they also want products to be processed in an environmentally friendly manner. Traditional antioxidant extractions have been performed with organic solvents. These solvents work on cells near the plant surface where anthocyanins are located. Commonly used solvents include aqueous mixtures of methanol, ethanol, and acetone, which are typically acid-

ified to improve anthocyanin stability. A comparative study of methanol, ethanol, and water acidified with various organic acids or HCl showed that methanol was the most effective solvent for extraction of anthocyanins from grape pomace followed by ethanol and water (Ju and Howard, 2003; Metivier et al., 1980). Organic acids and low concentrations of mineral acids improve the efficacy of organic solvents through the denaturization of cellular membranes of anthocyanin-containing cells. However, the amount of acid added must be well-controlled to prevent hydrolysis of sugar residues and acyl groups during subsequent concentration procedures (Jackman and Smith, 1996).

Research has shown that elevated extraction temperatures can improve solubility of analytes in solvents and speed diffusion rates (Ju and Howard, 2003). However, anthocyanins can degrade when exposed to elevated extraction temperatures. Thus, anthocyanin extraction is typically done at temperatures ranging from 20 to 50°C because temperatures greater than 70°C rapidly degrade anthocyanins and the expression of their pigments. Time and temperature determine rate of anthocyanin degradation. Therefore, extraction conditions consisting of high temperatures and short times are most successful in slowing anthocyanin degradation in fruits. Besides temperature, factors such as oxygen presence, metals, sugars, and light have been shown to affect stability of anthocyanins (Jackman and Smith, 1996).

Traditional organic solvents are expensive and potentially toxic, and thus present serious waste-disposal issues for industry (Ju and Howard, 2005). Therefore, industry retains an invested interest in subcritical-water extractions, where manipulations in temperature (>100°C) and pressure (>10 mPa) can be used to provide an environmentally sound and effective extraction procedure (Bakker et al., 1998). This method has been shown to be effective in extraction of biologically active compounds from a wide range of biological species such as antioxidants from rosemary and yams, essential oil from oregano, lignans from flaxseed, and ginsenosides from American ginseng (Cacace and Mazza, 2007). The basic concept of this method is that under the right conditions, an increase in the pressure of water can maintain water in its liquid state even after its boiling point is surpassed. The chemical properties of water are lowered in such a way that its dissociation constant, surface tension, viscosity, and polarity are closer to those of organic solvents than those of water at ambient -pressure conditions and temperature (Cacace and Mazza, 2007). Indeed, the presence of pressure enables a fast and efficient extraction of heat-sensitive compounds such as anthocyanins (Ju and Howard, 2005) that are normally degraded when held at high temperatures (>50°C) for extended periods of time (Jackman and Smith, 1996).

In order to confirm efficacy of subcritical water in extraction of anthocyanins from grape pomace, many extraction variables need to be tested and optimized. In this study, effects of pomace particle size and mass as well as solvent type and temperature on the extraction of antioxidant-rich anthocyanins from red grape pomace were studied.

MATERIALS AND METHODS

Red grape pomace (variety Sunbelt) obtained from the University of Arkansas wine-processing laboratory was lyophilized and ground in a Wiley mill (Thomas Scientific, Swedesboro, N.J., USA) to two particle sizes (ca 400 and 840 μm) by passing the material through 40- and 20-mesh screens. Powdered samples were stored in sealed brown vials at –20°C prior to extraction.

Subcritical water extraction of anthocyanins. A Dionex accelerated solvent extractor (ASE) Model 200 (Dionex Corp., Sunnyvale, Calif., USA) outfitted with a solvent controller was used for the study. Variables tested included mass of grape pomace (0.25, 0.5, and 1 g) and particle size (400 and 840 μm). Each sample was mixed with 29 g of sea sand and then placed into a 22 mL extraction cell containing a cellulose paper filter at the bottom of the cell. After addition of solvent, the extraction cell was pressurized and then heated. The ASE system was operated at zero extraction time and one extraction cycle. Approximately 5 to 7 min were needed to heat the sample/solvent from ambient temperature to the desired temperature, and then a short 40-sec static extraction was performed. Next, the cell was rinsed with 15.4 mL of fresh extraction solvent and purged from the extraction cell with a flow of nitrogen for 90 sec. To determine effects of extraction temperature on the recovery of anthocyanins, temperatures of 100, 110, 120, 130, and 140°C were tested using the basic ASE conditions described above, using Milli-Q-grade water (pH~7.3) and Milli-Q-grade carbonated water (sparged with CO_2 gas for fifteen min prior to use, pH ~3.6.) To prevent anthocyanin oxidation during extraction, solvents were sparged with nitrogen for two h prior to use. Samples were rapidly cooled following ASE extraction, adjusted to 50 mL with water, and stored at -20°C until analysis. Preliminary experiments of five extraction cycles showed that over 90% of anthocyanins were extracted within the first two extraction cycles and over 85% of anthocyanins were extracted within the first extraction cycle. All extractions were performed in triplicate.

Conventional solvent extraction of anthocyanins. For comparison with water and carbonated-water extractions, grape pomace samples (400 μm particle size) were

extracted with a solution consisting of methanol/water/formic acid at a ratio of 60:37:3 (v/v.) Samples (0.5 g) were homogenized for 1 min in 20 mL of extraction solvent, and then homogenates were filtered through Miracloth (CalBiochem, LaJolla, Calif., USA.) Filtrates were collected and centrifuged for 10 min at 2739 x *g*. Following centrifugation, supernatants of extracts were collected and adjusted to 50 mL with water. Extracts were stored at -20°C until analysis. All extractions were performed in triplicate.

Determination of total anthocyanins. Total anthocyanins were measured using the pH differential assay described by Giusti and Wrolstad (2001) using a Hewlett Packard 8425A photodiode array spectrophotometer (Palo Alto, CA, USA). The spectrophotometer was zeroed with distilled water at 510 and 700 nm. Two prepared dilutions, one with sodium acetate buffer at pH 4.5 and one with potassium chloride buffer at pH 1.0, were allowed to equilibrate for 15 min prior to the measurement of absorbance of each diluted sample consisting of 0.5 mL of sample and 4.5 mL of each buffer at 510 and 700 nm. Absorbance of the diluted sample was calculated by $A= (A_{510}-A_{700})_{pH\ 1.0}-(A_{510}-A_{700})_{pH\ 4.5}$ and monomeric pigment concentration in the original sample was calculated using the following formula: Monomeric anthocyanin pigment (mg/liter)=(A x molecular weight x dilution factor x 1000)/ (Molar absorptivity x 1). Molecular weight and molar absorptivity of malvidin-3-glucoside (m3g) were 492 and 28000, respectively. Results were expressed as mg of m3g equivalents per kg^{-1} dry weight.

Antioxidant capacity evaluation. Oxygen radical absorbing capacity ($ORAC_{FL}$) of extracts was measured using a FluoStar Optima microplate reader (Biomedical Solutions, Inc., Stafford, Texas, USA) using the method of Prior et al. (2003). Extracts from the first ASE extraction cycle were used for this assay. Grape pomace extracts were diluted 200-fold with phosphate buffer (75mM, pH 7) prior to ORAC analysis. The phosphate buffer prepared by combining 0.75M K_2HP_{O4} and 0.75M NaH_2PO_4 in a ratio of 61.6:38.9 (v/v) was diluted 1:9 (v/v) with DI water. A stock solution of fluorescein was prepared by dissolving 0.0225 g fluorescein (Sigma-Aldrich, St. Louis, Mo., USA) in 50 mL of 0.075M phosphate buffer. A second stock solution was made by diluting 50 µL of previously made stock solution with 10 mL phosphate buffer. A 1 mM Trolox (Sigma-Aldrich, St. Louis, Mo., USA) solution was prepared with phosphate buffer and stored at –20°C. The Trolox working solution was prepared by dilution of the Trolox stock solution to a final concentration of 50 µM with phosphate buffer. The Trolox working solution was diluted in phosphate buffer to obtain standard concentrations of 6.25, 12.50, 25.00, and 50.00 µM. Thereafter, 40 µL of diluted samples, trolox standards, and blanks (phosphate buffer) were manually pipetted into appropriate wells on 48-well microplates. A 320 mM solution of 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH; Wako Chemicals USA, Inc., Richmond, Va., USA) was prepared immediately prior to running the assay. Fluorescein solution was diluted (320 µL into 20 mL phosphate buffer) for the working solution. The FLUOstar Optima instrument equipped with two automated injectors was then programmed to add 400 µL of fluorescein followed by 150 µL of AAPH to each well. Fluorescence readings (excitation 485 nm, emission 520 nm) were recorded after addition of fluorescein, after the addition of AAPH, and every 192 sec thereafter for 112 min to reach 95% loss of fluorescence. Final fluorescence measurements were expressed relative to initial readings. Results were calculated based upon differences in areas under the fluorescein decay curves between blanks, samples, and standards. The standard curve was obtained by plotting four concentrations of trolox equivalents (TE) against net area under the curve (AUC) of each standard. Final ORAC values were calculated using the regression equation between TE concentration and AUC and were expressed as µmol TE per g dry weight.

Statistical analysis. Effects of pomace mass, particle size, and extraction temperature on the total anthocyanin content and antioxidant capacity of water, carbonated water, and conventional solvent extracts were analyzed by analysis of variance (ANOVA) using JMP® software (SAD Inst. Inc., Cary, N.C., USA). The Pearson correlation test was used to determine the correlation among total anthocyanins and ORAC values in water and carbonated-water extracts.

RESULTS AND DISCUSSION

Total anthocyanins (Fig. 1) generally decreased as extraction temperature increased. Subcritical water extracted the highest level of total anthocyanins over the 100 to 110°C temperature band, while subcritical carbonated water extracted the highest level at 100°C. At the optimal extraction temperature band of 100 to 110°C, water extracted 70% of the anthocyanins compared to the conventional methanol extraction method (1254.4±20.8 mg/kg DW), while carbonated water extracted 71% of anthocyanins compared to conventional methanol extraction.

The smaller particle size allowed for greater extraction of anthocyanins when solvent temperature was 100°C-130°C. At 140°C, there was no significant differ-

ence between the small and large particle size (Fig. 2). Maximum extraction of anthocyanins occurred at 100°C for the smaller particle size, while larger particle size achieved comparable extractions across the 100-110°C temperature band. Smaller particle size coupled with an extraction temperature of 100°C extracted 80% of anthocyanins compared to conventional methanol extraction. Presumably, smaller particle size allowed for greater contact with the solvent, which facilitated extraction of anthocyanins.

Carbonated water was more effective than water in extraction of anthocyanins for the small particle size (Fig. 3). However, when large particle size was used, both solvents achieved comparable extraction.

Use of the small particle size resulted in an approximate 64% extraction of anthocyanins compared to the conventional methanol extraction method for all sample masses tested (Fig. 4). However, extracts obtained with the larger particle size had higher amounts of anthocyanins with decreasing sample mass. Small particle size offered enough surface area to achieve the best extraction regardless of sample mass. The larger particle size with less surface area benefited from increased mass transfer obtained by a decrease in sample mass.

ORAC is an assay that determines total antioxidant capacity of samples, not solely the antioxidant capacity of anthocyanins. Total antioxidant capacity of subcritical-water and carbonated-water extracts increased as temperature increased (Fig. 5). According to statistical analysis, there was a significant inverse correlation between ORAC and Total Anthocyanins (r_{xy}=-0.29). This was most likely due to formation of antioxidant-rich Maillard reaction products when grape pomace was exposed to increasing temperatures. Compounds with antioxidant properties are formed during development of the Maillard reaction (Nicoli et al., 1997), and an increase in antioxidant capacity of foods typically occurs when they are heated at elevated temperatures due to the formation of brown melanoidin pigments formed during advanced stages of the Maillard reaction (Anese et al., 1999).

The general trend for particle size and temperature is that antioxidant capacity increased with increasing temperature for the large particle size. For the small particle size, there was no significant difference among temperature treatments (Fig. 6). Extracts obtained using smaller particle size had higher antioxidant capacities than extracts obtained using larger particle size. As with total anthocyanin results, increased surface area due to smaller particle size achieved greater antioxidant capacity than the large particle size.

Extracts obtained from samples with masses of 0.5 g and 0.25 g had similar ORAC values, while extracts obtained using a sample mass of 1.0 g had greater ORAC values at elevated extraction temperatures. This data group reinforces the trend that the greatest antioxidant capacity occurred at 140°C (Fig. 7).

Extracts obtained using small particle size had the highest ORAC value with a sample mass of 0.25 g, but antioxidant capacity decreased with increased sample mass (Fig. 8). Highest antioxidant capacity obtained with a sample mass of 0.25 g was 86% of the value obtained using conventional methanol extraction method (1191.3±18.1 μmol TE/g DW). Conversely, extracts obtained using large particle size showed a trend of increasing antioxidant capacity with increase in sample mass. Highest antioxidant capacity obtained using a sample mass of 1.0 g was 75% of the value obtained using conventional methanol extraction.

ACKNOWLEDGMENTS

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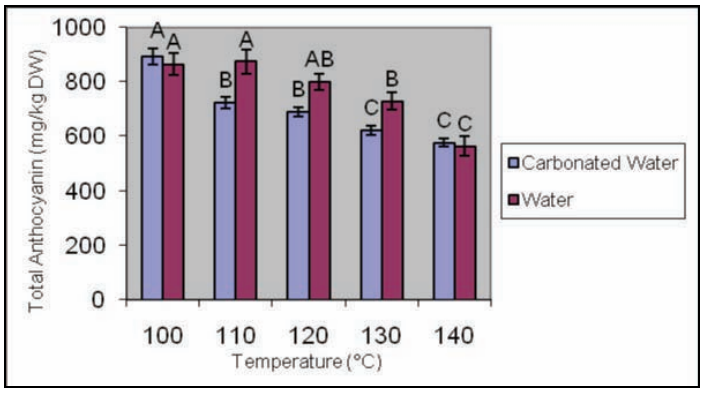
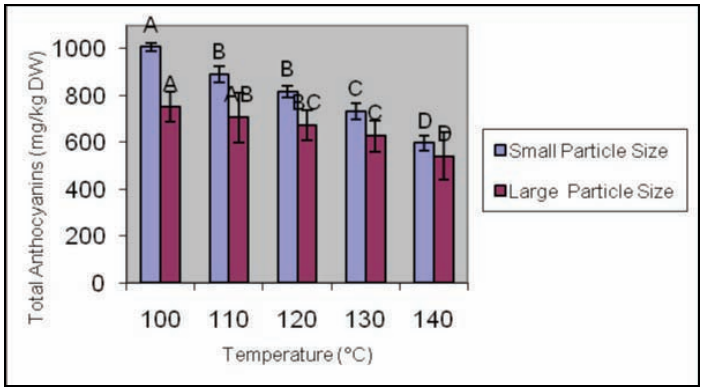


Fig. 1. Effects of temperature and solvent on total anthocyanins. Bars represent ± standard error of the mean (n=3). Bars with similar letters within each solvent are not significantly different (P>0.05).



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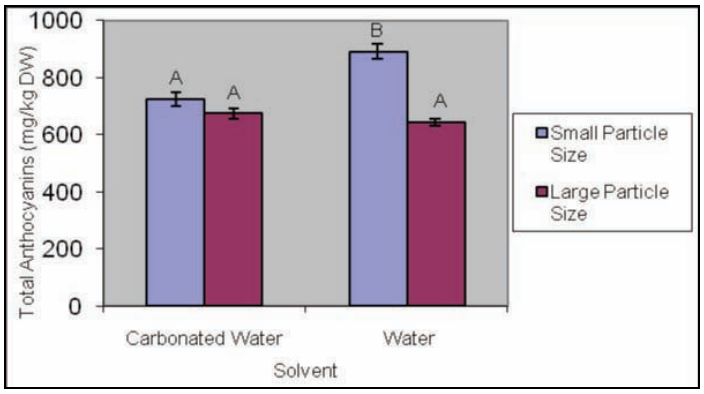

Fig. 3. Effects of particle size and solvent on total anthocyanins. Bars represent ± standard error of the mean (n=3). Bars with similar letters within each solvent are not significantly different (P>0.05).

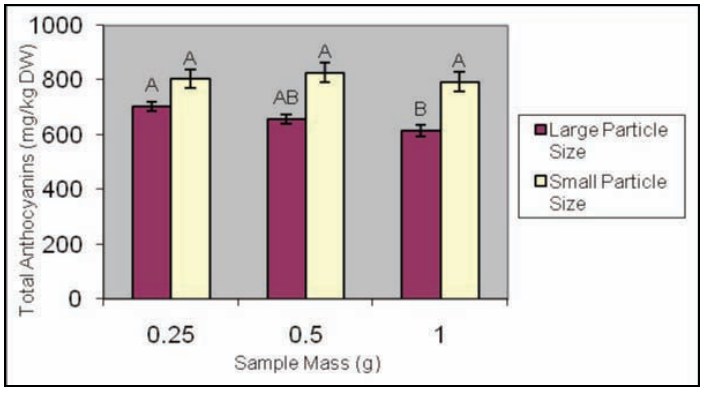

Fig. 4. Effects of particle size and sample mass on total anthocyanins. Bars represent ± standard error of the mean (n=3). Bars with similar letters within particle size are not significantly different (P>0.05).

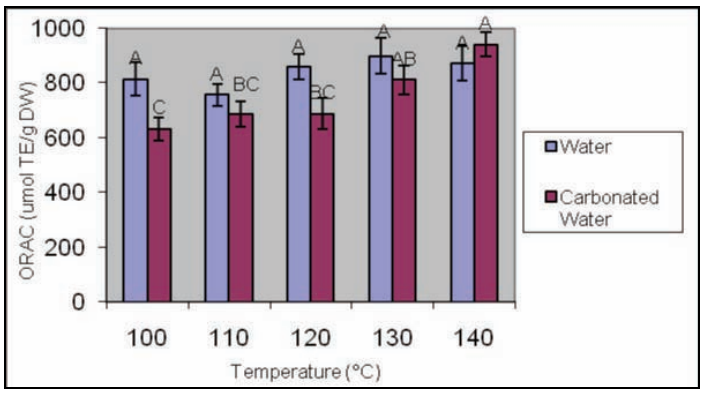

Fig. 5. Effects of temperature and solvent on ORAC. Bars ± standard error of the mean (n=3). Bars with similar letters within each solvent are not significantly different (P>0.05).

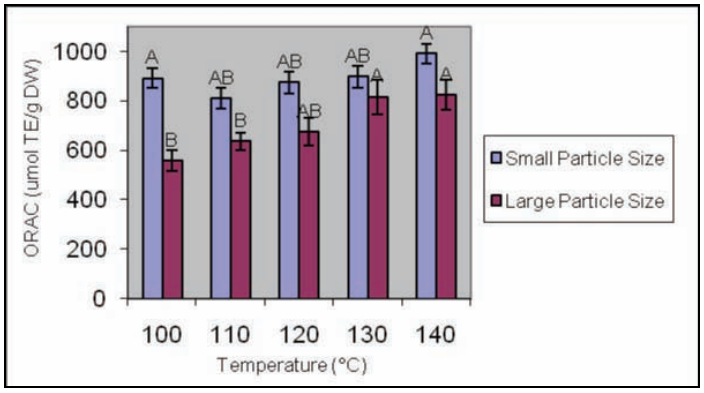

Fig. 6. Effects of temperature and particle size on ORAC. Bars represent ± standard error of the mean (n=3). Bars with similar letters within each particle size are not significantly different (P>0.05).

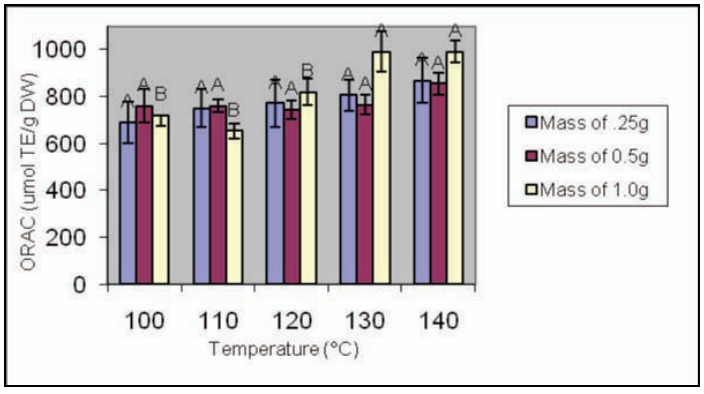

Fig. 7. Effects of temperature and sample mass on ORAC. Bars represent ± standard error of the mean (n=3). Bars with similar letters within each sample mass are not significantly different (P>0.05).

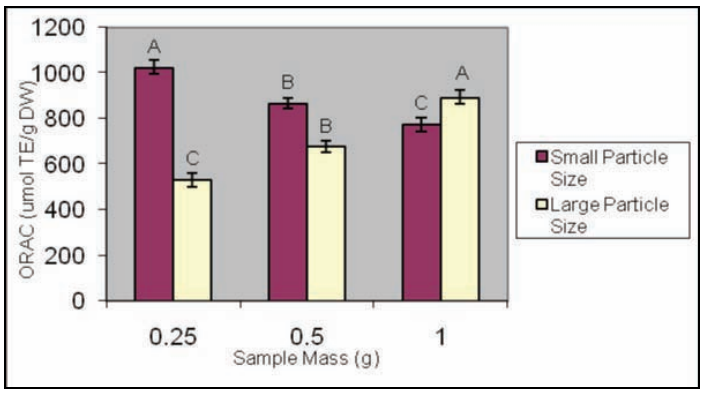

Fig. 8. Effects of particle size and sample mass on ORAC. Bars represent ± standard error of the mean (n=3). Bars with similar letters within each particle size are not significantly different (P>0.05).

Investigating current efficacies of several nematocides for use in cattle according to the fecal egg count reduction test

Tifanie Silver[*], *Chris Tucker*[†], *Jeremy Powell*[§], *Jana Reynolds*[‡], *Zelpha Johnson*[**], *Bill Lindsey*[††], *Pete Hornsby*[§§], *and T.A. Yazwinski*[‡‡]

ABSTRACT

Utilizing small groups of naturally infected replacement heifers, fecal egg count reduction tests (FECRT) were conducted in the later months of 2007 at the University of Arkansas Savoy Research Station. Each test was 28 d in length, consisting of individual fecal nematode egg counts (EPG) and coprocultures. For the first test, the calves were ranked by beginning EPG, blocked, and randomly assigned treatment within each block. Nine to ten animals were in each treatment group. In this test, neither IVOMEC (® Merial) or IVERMECTIN (® Durvet), both delivered as an injectable at the rate of 0.2 mg of ivermectin kg^{-1} BW, resulted in egg count reductions of ≥ 90%. Post-treatment coprocultures relative to both products contained a mixture of *Cooperia* and *Haemonchus* spp larvae. Also in this first test, Safe-Guard (® Intervet), delivered as a suspension at the rate of 5.0 mg of fenbendazole kg^{-1} BW, resulted in egg count reductions of 100% (d 7 and 14) and 88-87% (d 21 and 28). Post-treatment coprocultures specific to Safe-Guard yielded only *Cooperia* spp larvae. In the second test, which was of follow-up treatments given immediately after the first test (animals re-sorted to treatment group), Safe-Guard at the above rate resulted in egg count reductions of 99-100% (d 7 and 14) and 54-18% (d 21 and 28). Also in the second test, Cydectin (® Fort Dodge) treatment at the rate of 0.2 mg of moxidectin kg^{-1} BW resulted in egg count reductions of 96-92% (d 7 to 28) and Safe-Guard treatment at the rate of 10 mg of fenbendazole kg^{-1} BW resulted in egg count reductions of 100-88% (d 7 to 28). As was the case in the first test, post-treatment coprocultures from animals treated with Safe-Guard yielded only *Cooperia* spp larvae. Treatment of cattle with Cydectin resulted in coprocultures that primarily yielded *Cooperia*, but with a trace of *Haemonchus* spp larvae.

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INTRODUCTION

Infections of cattle by internal parasites may not be immediately apparent, but their potential detriment includes lowered weight gains, reproduction efficiencies, lactation, and forage utilization. In general, gastrointestinal nematodes of cattle share common life cycles. They are generally egg layers, male and female as adults, and follow a direct life cycle through four molts and six stages that are divided between the host animal and the environment (Yazwinski and Tucker, 2006). Eggs are shed in feces of an infected animal onto the grass. Once dung paddy stages of the parasites have developed for approximately 7 d, infective larval stages migrate up to 3 ft away from the paddy and are even able to ascend grass stalks. As larvae are consumed by cattle, they continue their maturation into adulthood within the gastrointestinal system and reproduce more eggs to be voided in the feces.

Regions of the U.S. that have hospitable conditions for raising cattle are also those that are optimal for nurturing parasite larvae on pastures (Yazwinski and Tucker, 2006). Of the approximately 26 species of gastrointestinal nematodes that infect cattle in the U.S., 10 are considered significant due to their prevalence and pathogenesis. Two particular genera of nematodes became the focus of this research project: *Haemonchus* and *Cooperia*. Species of the genus *Haemonchus* are most prevalent in southern regions of the U.S., and inhabit the abomasa of cattle. *Cooperia* species are very prevalent across the entire U.S. and inhabit the small intestine.

A major reason for persistence of these above nematodes is their resistance to many common anthelmintics. Most recent concern is over their resistance to macrocyclic lactones. In a recent study from New Zealand, it was determined that resistance extended to 92 percent of the nation's beef farms (Stafford 2007). Unfortunately, resistance to common anthelmintics has become worldwide (Kaplan, 2004). The two studies reported here provide additional information on ramifications of resistance in cattle. In the trials, efficacies of popular anthelmintics were evaluated by conducting fecal egg count reduction tests—a universally accepted means of documenting anthelmintic effectiveness.

MATERIALS AND METHOD

Animals and study initiation. A group of 30 naturally infected heifers weighing approximately 270 kg was assembled at the University of Arkansas Beef Unit in Savoy, Ark. A week prior to the beginning of the first trial (d -7) fecal samples were obtained for the determination of fecal nematode egg counts (EPG) of a 1 g sample of feces using direct $MgSO_4$ flotation and centrifugation (Ives, et al, 2007). Animals were then ranked in order of their egg count magnitudes. Using this ranking, animals were blocked into replicates and then randomly assigned treatment within replicate. Ten animals were assigned to each treatment group. Throughout this investigation, all heifers were kept on pasture.

First trial. In the first trial, Ivomec (® Merial), Ivermectin (® Durvet), and Safe-Guard (® Intervet) were evaluated via a fecal egg count reduction test (FECRT). Ivermectin-containing products were delivered at a rate of 0.2 milligrams per kilogram of body weight (MPK) as a subcutaneous injection. Safe-Guard was delivered as an oral suspension at a rate of 5 mg fenbendazole per kg BW. On d 0, all heifers were fecal sampled, weighed for proper dosage, and treated. Fecals were taken again on d 7, 14, 21, and 28 post-treatment. Fecal samples with an EPG ≥10 eggs were coprocultured to obtain infective larvae. For these coprocultures, feces were put into 10-oz cups and mixed with crushed corncob, stored for 14 d at room temperature and the resultant parasite larvae harvested by flooding (Roberts, 1949). The larvae were subsequently killed with formaldehyde and stretched with heat, for identification at 40-100 X. Pictures of representative larval sheath tails seen in this study are given in Figure 1.

Second trial. At the end of the 1st trial, the animals were once again ranked by egg count (day 21) and reassigned new treatments randomly in each replicate. On d 28 of the first trial (designated d 0 of the second trial) heifer weight was again obtained for proper dosage and treatments were administered. Safe-Guard was delivered at 5 and 10 MPK, and moxidectin (Cydectin® Fort Dodge) was delivered as a subcutaneous injection at 0.2 MPK. On d 7, 14, 21, and 28 of the second trial, fecal samples were taken again for EPG's and coprocultures, using the same methods as described in the first trial.

Statistics. All egg counts were transformed to the $\log_{10}(x + 1)$ prior to analysis of variance. This transformation is commonly used when analyzing data with high animal-to-animal variance (SAS, Carey, N.C.). Fecal egg count reduction percentages were calculated with treatment group, geometric means (each post-treatment day versus day of treatment).

RESULTS AND DISCUSSION

From egg count reductions seen in the first trial (Fig. 2), it is apparent that Safe-Guard was more effective than either ivermectin-containing treatment. *Cooperia* and *Haemonchus* spp were the most predominate genera of larvae harvested from the coprocultures prior to animal treatment. Heifers treated with Ivomec or Ivermectin

Tifanie Silver

MEET THE STUDENT-AUTHOR

I came to the University of Arkansas on a recruitment trip in spring 2004 and decided that, after meeting with members of the Animal Science Department, this was the place for me. I enrolled the following fall semester as an animal science major with a focus in pre-veterinary medicine. I am originally from Honolulu, Hawaii. After moving to Dallas, Texas, in 1996 at the age of 10, I began swimming. I was recruited by the University of Arkansas on a swimming scholarship in 2004, and as a senior I became a co-captain for the team. I attended three SEC Championships and in our third appearance we finished 7th overall. I have been placed on the SEC Academic Honor Roll and competed in the 2008 Gamma Sigma Delta Undergraduate Oral Competition, placing 3rd. I have participated in community service projects associated with the Lady Razorbacks Athletics as well as the Arkansas Athletes Outreach program. I plan to continue my education in graduate school at the University of Arkansas in pursuit of my goal of gaining entry to veterinary school.

retained patent (mature, egg-laying) infections of both genera of nematode (Figures 3 and 4). For heifers treated with Safe-Guard, only *Cooperia* egg shedding was continued after treatment.

In the second trial, Cydectin and Safe-Guard (10 MPK) treatments were both effective to d 28 post-treatment (Fig. 5). At 5 MPK, Safe-Guard reduced egg counts significantly for only 14 d. As in the 1st trial, only *Cooperia* eggs were voided after fenbendazole use (as determined by coproculture). For Cydectin, *Cooperia* with a trace of *Haemonchus* larvae were seen post-treatment.

Based on the results from the first trial, it is apparent that neither the original nor generic forms of ivermectin provided ≥90% reductions of egg counts in treated heifers—a threshold established for acceptable efficacy (Coles, Jackson, Pomroy et al, 2006). Coproculture results indicate that both *Cooperia* and *Haemonchus* spp remain viable and patent after animal treatments with ivermectin. As an initial or "clean-up" anthelmintic, Safe-Guard was ≥90% effective in fecal egg count reductions. As noted out to 14 d after treatment, Safe-Guard at 5 MPK is much better as an initial treatment as opposed to a "clean up." This may be due to the existence of nematode populations that have become resistant to Safe-Guard at the 5 MPK dose rate, but not the 10 MPK rate. Cydectin, with the active ingredient of moxidectin, was ≥90% effective during the entire 28-d "clean up" test period.

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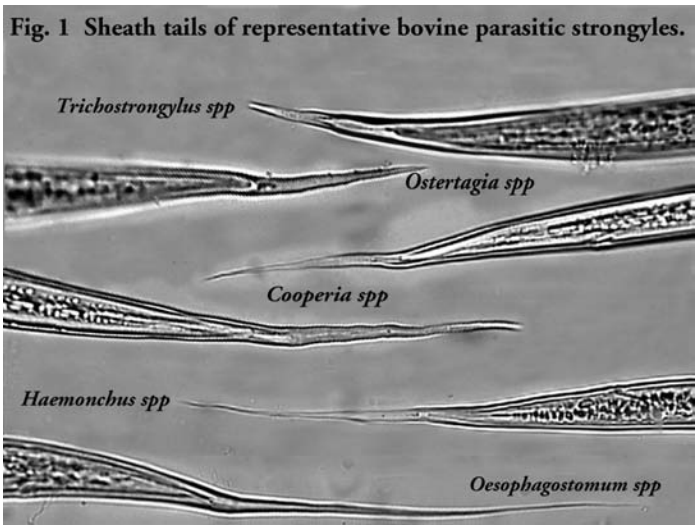

Fig 1. Sheath tail of representative bovine parasitic strongyles

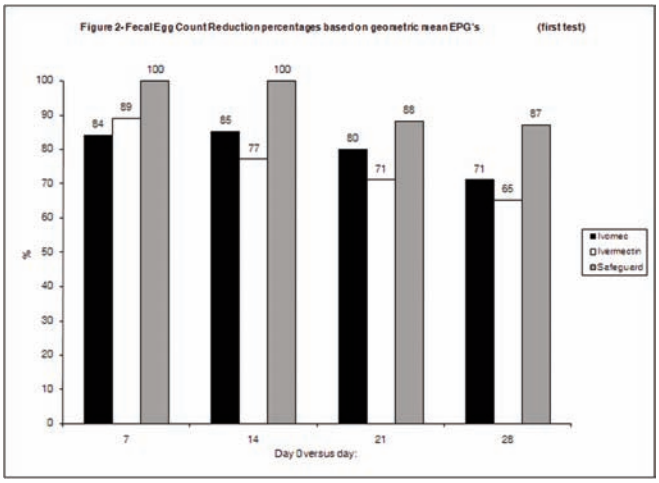

Fig 2. Fecal egg count reduction percentages based on geometric means (first test)

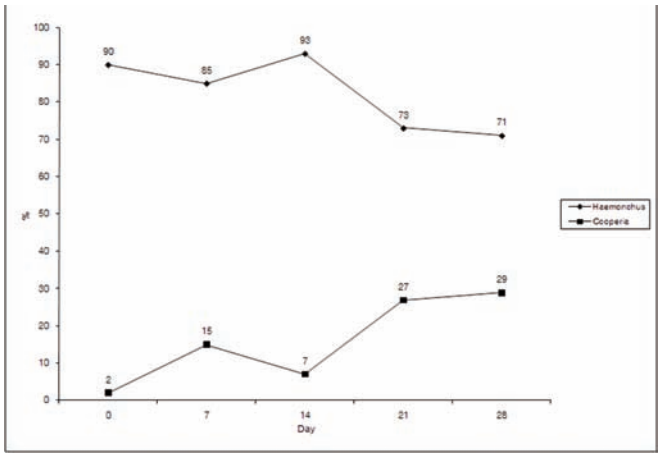

Fig 3. Coproculture larvae percentages for IVOMEC in the first trial

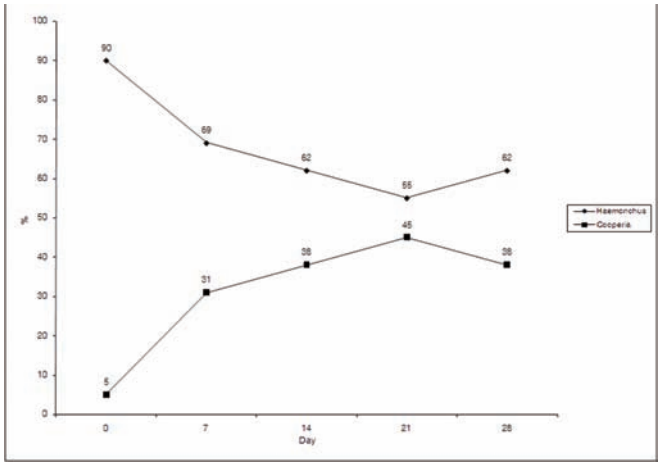

Fig 4. Coproculture larvae percentages for IVERMECTIN in the first trial

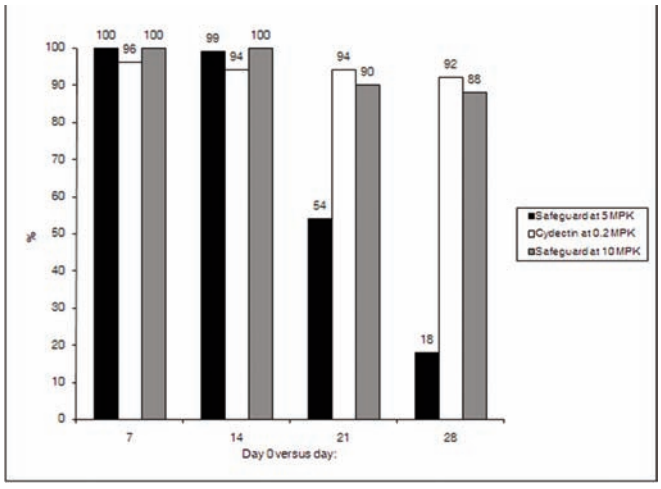

Fig 5. Fecal egg count reduction percentages based on geometric mean EPGs (second test)

Investigating the utilization of silica gel packets in drying research-scale rough rice samples

Ashley Wiedower[*]*, George Ondier*[†]*, and Terry Siebenmorgen*[§]

ABSTRACT

Rice moisture content (MC) must be reduced to approximately 12.5% MC to prevent spoilage during storage. Desiccants may provide an improved method for drying research-scale rice samples. This study investigated the effects of 1) rice mass to be dried, 2) placement method of silica gel packets in rice samples, 3) regeneration and re-use of the packets, 4) drying temperature, and 5) initial MC on the effectiveness of silica gel packets to dry rough rice samples to the desired 12.5% MC. Multiple masses (200, 500, and 1000 g) of long-grain rice samples were dried using three desiccant placement treatments: 1) intimate mixing (IM) of silica gel packets without agitation, 2) intimate mixing and agitation (IMA), and 3) surface placement (SP) of silica gel packets on top of the rice samples. The IMA treatments produced little variability in final MCs across the three masses used. The adsorptive capacity of silica gel packets in 200-g samples of two rice cultivars was measured. The adsorptive capacity varied from 26 to 35%. Effects of rice initial MC and drying temperature were measured by drying samples at initial MCs from 13 to 18% at 10°C, 20°C, and 30°C for eight days. Increased drying temperatures produced decreased final MCs for both cultivars, which became more pronounced as the initial MCs increased.

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[†] George Ondier is a Department of Food Science graduate assistant at the University of Arkansas.

[§] Terry Siebenmorgen is a university professor in the Department of Food Science at the University of Arkansas.

Ashley Weidower

MEET THE STUDENT-AUTHOR

I graduated in May 2004 from Mount Saint Mary Academy in Little Rock, Ark., with an International Baccalaureate Diploma. After graduation I enrolled at the University of Arkansas with the intent of pursuing a degree in biological engineering. I was awarded the University Scholarship, Department of Biological Engineering Scholarship, and a General College of Engineering Scholarship. Throughout my college career, I have served as treasurer of Engineers without Borders, scholarship chair for Alpha Delta Pi Sorority, and as a mentor for the Society of Women Engineers.

My interest in food-related engineering applications began following a class I took through the Biological and Agricultural Engineering Department called bioreactor design. I was fascinated by the number of physical, chemical, and biological properties that must be considered when designing food-processing equipment. I received the opportunity to learn more about the food industry when I began a special problems project with Dr. Siebenmorgen in the Food Science Department in spring 2007. I have thoroughly enjoyed my research experience, and this opportunity has allowed me to develop my research and technical skills, which will prove beneficial in the future. I plan to enroll in the Department of Food Science graduate school in fall 2008, and one day, work as a food-processing engineer at a major food corporation.

INTRODUCTION

In the mid-South rice growing regions of the United States, rough rice is generally harvested at 14% to 22% moisture content (MC*) (Schluterman et al., 2004). Breeders and other rice scientists annually harvest large numbers of small rice samples from different cultivars and at varying MCs. To minimize microbial activity and respiration, and thus establish a safe storage environment, the rice MC must be reduced to less than 14% (Schroeder, 1963; Dillahunty et al., 2000).

Methods currently used for drying small rough rice samples involve exposure to air at low temperatures (< 40°C) and relative humidities (RHs) (<60%) until the desired final MC is attained. Higher drying temperatures, typical of those used in commercial driers, are reported to have a negative impact on grain quality, especially germination (Danziger et al., 1972), which makes these commercial methods unsuitable for drying research-scale samples (Aldis et al., 1980). Drying these samples in air-conditioned rooms often results in large variation in final MCs, which subsequently leads to variation in processing property measurements.

One possible solution to minimize final MC variation involves the use of desiccants. Desiccants have been used successfully to dry inshell pecans (Ghate and Chinnan, 1984). Yamaguchi and Kawasaki (1994) modeled two methods of drying rice with silica gel, a common desiccant that has potential in grain dying. Use of solar-regenerated silica gel to dehumidify air was found to significantly reduce the drying duration for corn and milo (Aldis et al., 1980). Danziger et al. (1972) determined that corn dried with desiccated air increased germination rates. Desiccants have also been used to reduce MC of soybeans (Zhangyong et al., 2002), corn (Graham et al., 1983), wheat and oats (Sturton et al., 1983) to levels safe for storage.

Silica gel is inert, has a high absorbency, and can be regenerated easily using high temperatures (>100°C) without significant reduction in adsorptive capacity (Koh, 1977). Silica gel is available in various-sized, moisture-permeable packets that would be ideal for drying small samples of rough rice. Such packets offer excellent handling properties, reduce separation costs, and minimize the risk of product contamination.

To fully understand sorption drying of rough rice using silica gel, a thorough knowledge of the moisture transfer relationships between silica gel and rough rice is

required. In particular, measuring the equilibrium MCs of rice and silica gel is critical because final MC, and not drying rate, is influenced by the silica gel-to-grain mass ratio (Sun and Woods, 1997). Information on the adsorptive capacity and the effect that repeated regeneration cycles have on silica gel effectiveness is also required to evaluate potential use of silica gel in rice drying (Ghate and Chinnan, 1984).

The goal of this study was to investigate an alternative to conventional air drying of research-scale rough rice samples. It was therefore important to determine the parameters and conditions that may alter adsorptive behavior of silica gel packets and to establish a set of guidelines for use of the packets. Objectives were to a) determine influence of the mass of rice dried and method of placement/mixing packets in rough rice samples on drying effectiveness, b) determine adsorptive capacities of new and regenerated silica gel packets in rough rice samples, and c) determine effect of drying temperature and initial rice MC on achieving a desired, final MC of 12.5%.

MATERIALS AND METHODS

The first objective was to determine optimal desiccant placement, sample treatment, and rice mass to be used to maximize drying effectiveness of silica gel packets. The second objective sought to quantify the mass of desiccant required to dry a rice sample to a target MC of 12.5% and to determine if silica gel packets could be regenerated without a reduction in adsorptive capacity. The final experiment investigated effects of air temperature and initial rice MC on final MC of rice samples.

Rice sample preparation. 'Wells' and 'CL730' rice cultivars (long-grains) harvested in 2007 from Stuttgart, Ark. at 17.1% and 20.2% MC, respectively, were cleaned using a dockage tester (XT4, Carter-Day Co., Minneapolis, Minn.) and stored in covered, 32-gallon plastic bins at 5°C for 12 wks. Bulk sample MCs were determined after harvesting and before storage by drying duplicate 20-g rice samples in a convection oven (1370 FM, Sheldon Inc., Cornelius, Ore.) maintained at 130°C for 24 h (Jindal and Siebenmorgen, 1987). Before each test, the samples were removed from cold storage and equilibrated to room temperature while in plastic bags to prevent condensation onto the samples.

Rice sample treatment and desiccant placement. Three drying methods were investigated: intimate mixing (IM) of rough rice and silica gel packets without agitation, intimate mixing and agitation (IMA) of the drying container at 24-h intervals over the drying duration, and surface placement (SP) of silica gel packets on top of the rice samples. Effect of rough rice sample mass on each drying method was also investigated. Samples of 'CL730' rice, initially at 20.7%, were divided into 200-, 500-, and 1000-g denominations and dried using 5-g silica gel packets by each of the three above methods (Fig. 1). Each rice sample, with its corresponding mass of desiccant, was placed inside a re-sealable (Ziploc) plastic bag.

To determine the mass of silica gel packets needed to dry these samples to the target MC of 12.5%, the adsorptive capacity of the silica gel packets was estimated to be 25% (0.25 g of H_2O/1 g of silica gel). The mass of silica gel required was dependent on the mass and initial MC of the rice to be dried and was the same for each of the three drying methods. For instance, drying 200 g of rice from an initial MC of 20.7% to the desired 12.5% MC required the removal of 18.7 g of water (determined by mass balance). The mass of silica gel necessary was then determined by dividing the mass of water to be removed by the estimated adsorptive capacity of the packets. Given an estimated adsorptive capacity of 25%, 75 g of silica gel (18.7 g of H_2O/0.25 g H_2O/g silica gel) would be required to dry the rice samples to the desired final MC of 12.5%. Thus, fifteen 5-g packets and zero 1-g packets were used. In situations where the required silica gel mass was not evenly divisible by five, in which a fraction of a 5-g packet would be needed, a combination of 1- and 5-g packets was used to supply the required mass and thereby minimize the possibility of over- or under-drying the rice samples.

The IMA samples were mixed by manually shaking the plastic bags containing the rice samples and silica gel packets at 24-h intervals to improve air circulation, thus increasing the mass transfer rate from the rice kernels to the inter-particle air and subsequently to the silica gel packets. Theoretically, it was assumed that the silica gel-packet adsorptive capacity would be optimized and the drying rate maximized if the plastic bags containing the rice samples and silica gel packets were periodically agitated. The IM samples were not agitated except at the beginning of the experiment. The SP drying method comprised placing silica gel packets directly on top of the rice bulk; the samples were not agitated. All plastic bags remained sealed and were kept in a chamber maintained at 26°C for 8 d, after which the packet masses were measured using an analytical balance with an accuracy of ± 0.0001 g (EO1140, Ohaus Co., Zurich, Switzerland). The rice MCs were determined by the oven method previously described. The actual adsorptive capacities of the packets were determined as the mass percentage increase of the initial desiccant-packet mass.

Adsorptive capacities of new and regenerated silica gel packets. Silica gel adsorptive capacity (as indicated by the manufacturer) is usually based on exposure to a moisture-saturated environment and is typically expressed as

mass of water adsorbed per mass of desiccant. Adsorptive capacity in closed samples of rough rice is expected to be lower due to resistance to moisture migration inside kernels. To determine the maximum adsorptive capacity of the silica gel packets, twenty 1- and 5-g silica gel packets (ten of each) were placed on a wire mesh in a closed metal container that was partially filled with water such that only moisture-saturated air came in contact with the silica gel packets. The packets were held in this saturated environment at 26°C for 8 d. The initial and final masses of each packet were measured as previously described, after which the packets were regenerated in a convection oven (1370 FM, Sheldon Inc., Cornelius, Ore.) at 130°C for 24 h. The regenerated packets were then re-exposed to the saturated environment for 8 d (Fig. 2). The exposure and regeneration procedure was repeated twice in order to establish the maximum adsorptive capacity of the new, once-, and twice-regenerated silica gel packets studied. The loss of effectiveness of the packets resulting from subsequent regeneration was determined by the reduction in the adsorptive capacity of the regenerated packets compared to the adsorptive capacity of the new packets. Each experiment was replicated.

As a means of estimating the actual adsorptive capacity of the silica gel packets in rough rice, a procedure was developed in which assumed adsorptive capacities ranging from 15 to 30% were used to calculate the mass of desiccant required to reduce rough rice samples to the desired 12.5% MC. After drying, a graph of the final rice MCs against the assumed adsorptive capacities was plotted, and a regression line was used to indicate the adsorptive capacity that corresponded to the desired 12.5% final rice MC (Fig. 4). Eight, 200-g samples (two replicates) each from 'CL730' and 'Wells' cultivars, initially at 20.2% and 17.1% MC, respectively, were dried using new 5-g packets (Fig. 2). The mass of desiccant placed in each sample was determined based on the assumed desiccant adsorptive capacities (15% to 30%) and the mass balance procedure previously described. The rough rice samples and silica gel packets were intimately mixed (IM) in plastic bags and kept in a chamber maintained at 26°C for 8 d. The mass of the silica gel packets and MCs of the rice samples were determined using the previously-described methods. The silica gel packets were regenerated in the same convection oven at 130°C for 24 h, and then re-used to dry a second batch of rough rice from the same initial MCs following the same procedure (Fig. 2). The regeneration and drying cycles were repeated twice.

Initial MC and temperature effects on final rice moisture content. Bulk rice samples from the 'CL730' and 'Wells' lots, initially at 20.2 and 17.1% MC, respectively, were conditioned in a chamber maintained at 26°C and 55% RH for varying durations to yield samples at the target initial MCs of 18%, 15%, and 13%. Duplicate 200-g samples from each cultivar and conditioned MC lot were then dried in plastic bags using 5-g silica gel packets in chambers maintained at 10°C, 20°C, or 30°C for 8 d (Fig. 3) to determine the influence of initial MC and drying temperature on final rough rice MC. For this experiment, the silica gel adsorptive capacity was estimated as 25%. The initial and final rice sample MCs were determined using the above oven method.

All statistical analyses, which included analysis of variance (ANOVA) and linear regression, were performed using JMP 7.0.1 (SAS Inst. Cary, NC).

RESULTS AND DISCUSSION

Rice sample treatment and desiccant placement. Based on results of a T-distribution analysis, the mass of rice dried produced significant effects (p-value < 0.05) on the silica-gel adsorptive capacities for the surface placement (SP) and the intimately mixed (IM) treatments. Significant differences (p-value 0.0146) for the SP treatment were found between the 500- and 1000-g samples, whereas there were no significant differences between the 200- and 500-g samples (Table. 1); adsorptive capacities of the 200-, 500-, and 1000-g samples were 25.5, 24.1, and 17.8%, respectively. This trend is attributed to reduced interaction between the silica gel packets and rough rice kernels, after a certain mass of rice was reached. By placing the silica gel packets on the surface of the rice bulk, the effective packet surface area in contact with the rice was reduced, thereby increasing the resistance to moisture migration from the kernels. This lack of direct interaction between the silica gel packets and rough rice kernels contributed to the slow rate of moisture diffusion into the silica gel packets, which became more evident as the mass of rice dried using this treatment increased.

Within the intimately mixed and agitated (IMA) samples, adsorptive capacities of the silica gel packets in the 200-, 500-, and 1000-g samples were not different (p-value >0.05), and therefore, mass of rice dried did not influence the resulting adsorptive capacities of the packets. The increased interaction between the rice kernels and desiccant packets due to the daily agitation with the IMA method minimized the effects of rice mass on the adsorptive capacities for this treatment.

Significant differences (p-value 0.0748) for the IM treatment were found between the 200- and 500-g samples, whereas there were no significant differences between the 200- and 1000-g samples or between the 500- or 1000-g samples. Adsorptive capacities for the

200-, 500-, and 1000-g samples were 26.3, 23.1, and 24.4%, respectively. Even though the desiccant packets were supposedly distributed evenly throughout the rice samples in the IM method, there may have been some incomplete mixing in the 500-g sample; this incomplete, initial mixing would be alleviated by the practice of daily agitating the samples in the IMA method.

Upon examining the final MC for each mass denomination and drying method, there was a consistent, inverse correlation between adsorptive capacity and final rice MC. Thus, the trends in final MC due to drying method and rice mass reflect that of the desiccant adsorptive capacity. The IM 200-g and the IMA 200-, 500-, and 1000-g rice samples were dried to MCs below the desired 12.5% (Table 1), which corresponds to the greater adsorptive capacities previously recorded in these samples (> 26%). These over-dried samples indicate that the mass of desiccant present in those samples was more than that required to reach the 12.5% target MC. Since the required desiccant mass is inversely proportional to the adsorptive capacity of the desiccant, the actual adsorptive capacity of these packets was effectively greater than the initially estimated value of 25%. This same trend is illustrated with the final MCs of the rice samples and adsorptive capacities of rice dried using the SP method, wherein the rough rice samples may have retained a greater amount of moisture due to less and non-uniform osmotic pressure developed within the plastic bag.

In summary, the average adsorptive capacity of the silica gel packets was dependent on the level of sorption interaction between the silica gel packets and rough rice. Intimately mixing and agitating the rice with the silica gel packets optimized the packets' adsorptive capacities due to increased interaction between the silica gel and the rice kernels. Surface placing the packets on the rice samples was the least efficient method of drying, yet with the 200-g masses, the SP method produced statistically similar adsorptive capacities as the other two methods, indicating that this method is suitable for small samples, but unsuitable for larger masses of rice. Comparing the IM and IMA methods showed some inconsistency was found in adsorptive capacities and final MCs if samples were not agitated. From a practical standpoint, little difference in final MCs was observed between the IM and IMA methods for the 200-g samples; for greater sample masses, periodic agitation may be needed to attain maximum drying.

Adsorptive capacities of new and regenerated silica gel packets. While there were no significant differences (p-value 0.7834) observed between the maximum adsorptive capacities of the new, once-, and twice-regenerated 1-g or 5-g silica gel packets exposed to an environment saturated with moisture, there was an apparent increase in the adsorptive capacities when regenerating the new desiccant packets for the first time; this increased capacity remained constant for the subsequent regeneration (Table 2). Due to opening and closing of the desiccant container in which the packets were stored, new packets were repeatedly exposed to moisture in the air, which may account for the greater initial mass of the new 5- and 1-g packets in comparison to the once-regenerated packets and the corresponding increased adsorptive capacities. Since the once- and twice-regenerated packets were both oven-dried to remove moisture adsorbed in the saturated environment, the initial desiccant masses of both treatments were equal. Consequently, the final average adsorptive capacities were also similar. This observation could have implications for the procedure used by practitioners since the regeneration process appears not to have an effect on the adsorptive capacities of silica gel packets. It is also important to note that while there were no statistical differences between the adsorptive capacities of the 1-g and 5-g packets, the 1-g packets were consistently lower than the capacities of the 5-g packets.

A regression analysis was used to determine the actual adsorptive capacity of the silica gel packets when drying rough rice (Fig. 4). Actual adsorptive capacity was determined as the value on the x-axis that corresponds to the desired 12.5% rice MC on the y-axis. The adsorptive capacity of the new silica gel packets needed to dry 'Wells' samples initially at 17.1% was greater (35.4%) than that required to dry 'CL730' samples initially at 20.2% MC (28.7%). Since both rice cultivars were exposed to the same drying temperature, the initial MC of the rice may account for the adsorptive capacity differences between the CL730 and Wells cultivars studied.

After these same silica gel packets were regenerated once, the adsorptive capacity from the regression analysis for the 'CL730' cultivar increased slightly from 28.7% to 30.2%, while the corresponding analysis for the 'Wells' cultivar produced a slight decrease in adsorptive capacity from 35.4% to 34.5% (Table 3). Adsorptive capacities of the twice-regenerated silica gel packets revealed a significant drop between the once- and twice-regenerated packet adsorptive capacities. The adsorptive capacity for twice-regenerated packets in the 'CL730' samples was considerably lower (26.5%) than the adsorptive capacities of the once-regenerated desiccants (30.2%), as were also the twice-regenerated and once-regenerated desiccant adsorptive capacities for the 'Wells' cultivar (30.9% and 34.5%, respectively).

An additional experiment and regression analysis were performed based on assumed adsorptive capacities ranging from 15 to 45% to better span the actual adsorp-

tive capacity range of 25 to 35%. This analysis provided results (Table 3) similar to the initial regression analysis in that the adsorptive capacity of 'Wells' (33.7%) was greater than that of 'CL730' (26.5%). The adsorptive capacity values from this analysis were slightly less than initial new desiccant analysis values. This experimental verification, based on a range of adsorptive capacities from 15 to 45%, was performed several weeks after the initial experiment began. As previously discussed, due to opening and closing the desiccant storage container, desiccant packets were exposed to the surrounding air allowing the packets used in the 15 to 45% analysis to adsorb more moisture than those used in the 15 to 30% analysis. The inconsistent results of this experiment merit further testing.

Effect of initial MC and temperature on adsorptive capacity. The effects of initial MC and ambient temperature on the final MCs of samples from the two cultivars conditioned to various initial MCs are shown in Figure 5. Ambient temperature had a similar effect on the final MCs of both cultivars in that as the drying temperature increased, the final MCs decreased. The initial MC of a sample also affected its final MC. The difference between the final MCs of samples at the same initial MC and from the same cultivar increased as the initial MC increased. The final MCs for both cultivars were most likely determined by rice equilibrium MC trends in that as grain temperature increases, the equilibrium MC decreases, causing rice at higher drying temperatures to reach a lower final MC.

When comparing samples dried at the same temperature, the final MCs of the Wells samples were lower than those of the CL730 samples at similar initial MCs, which may be due to differences in the internal kernel matrices and constituents of each cultivar. These internal kernel differences were also believed to influence the final MCs in the previous section, which determined the actual adsorptive capacity of each cultivar.

In summary, as ambient temperature increased, the final MCs decreased for both cultivars, as would be expected due to equilibrium MC trends. Differences in final MCs associated with the drying temperatures became more pronounced as the initial MCs increased. The initial MCs, however, were not the sole determinant of the final MCs at a given temperature, indicating inherent differences in the final MCs associated with the cultivars. The practical ramification of this is that to achieve a 12.5% final MC, differences in adsorptive capacities may be required for various cultivars, or, if the same adsorptive capacity is used across cultivars, some inherent cultivar-to-cultivar variability in final MC may be expected.

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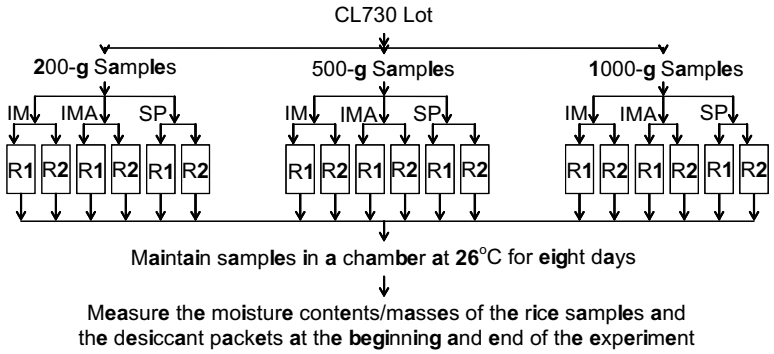

Fig. 1. Schematic of the experiment to determine effect of intimately mixing rice samples with silica gel packets (IM), intimately mixing with agitation (IMA) and surface placing (SP) packets above rice bulk, on adsorptive capacity of 5-g silica gel packets.

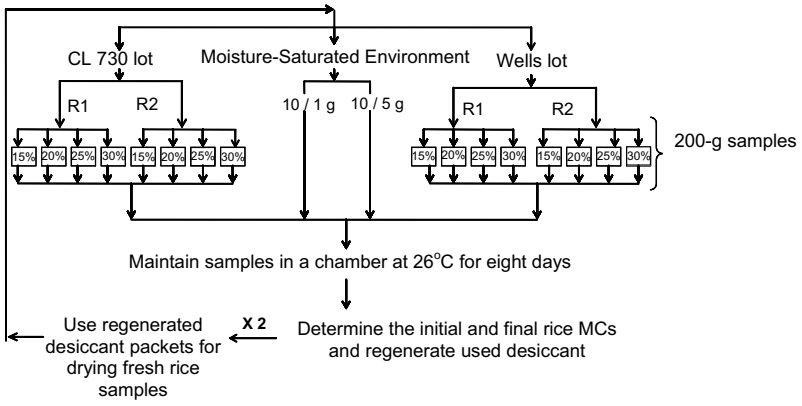

Fig. 2. Schematic of the experiment to determine adsorptive capacities of new and regenerated silica gel packets in an environment saturated with moisture and intimately mixed in rough rice using assumed adsorptive capacities of 15 to 30%.

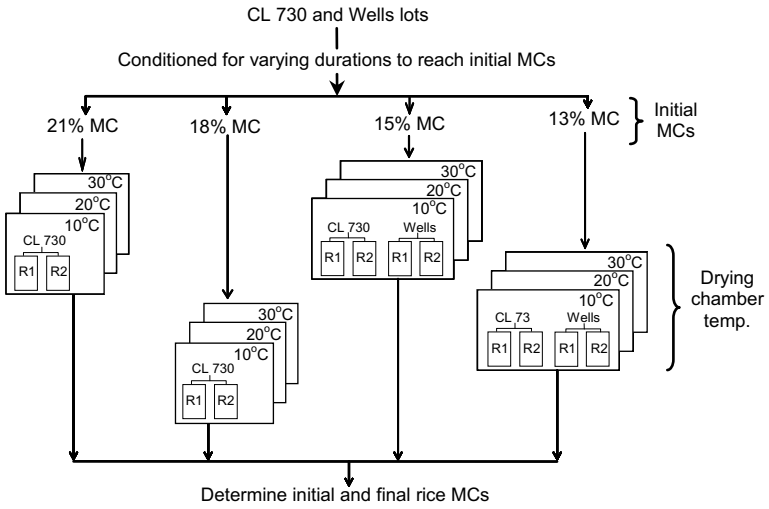

Fig. 3. Schematic of the experiment to determine effect of temperature and initial moisture content (MC) on final rice MC when drying Wells and CL730 rice samples using silica gel packets.

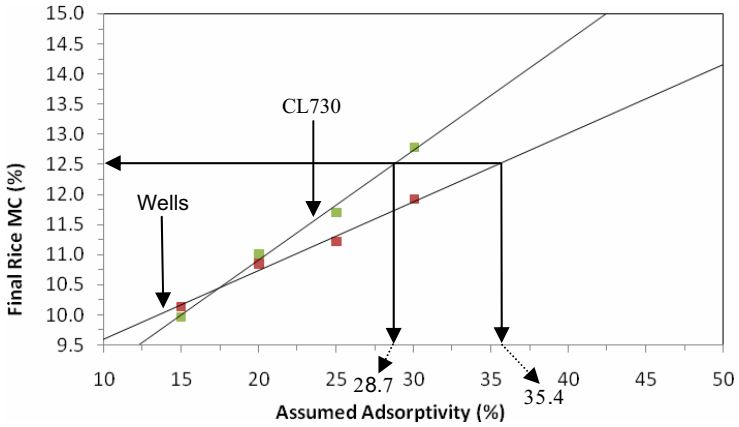

Fig. 4. Linear regression used to determine actual adsorptive capacity of new silica gel packets needed to dry intimately mixed rice samples of Wells and CL730, initially at 17.1 and 20.2% MC, respectively, to a desired 12.5% MC within a period of 8 d. Each data point represents an average of four measurements.

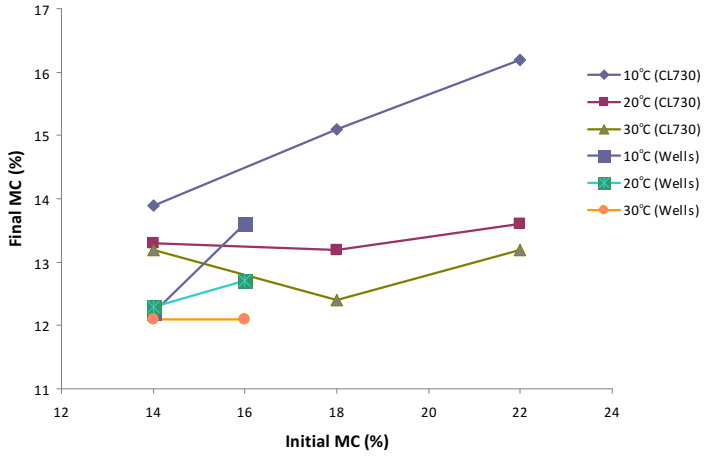

Fig. 5. Linear analysis evaluating effect of temperature on final moisture content (MC) of CL730 and Wells cultivars when conditioned to a given initial MC.

Table 1. Comparison of adsorptive capacities of silica gel packets and final rice moisture contents (MCs) as influenced by mass of rice dried and drying method. Initial adsorptive capacity of silica gel packets was estimated as 25%. Values not listed with the same letter are significantly different.

Drying Method	Rice Mass (g)	Initial Desiccant Mass (g)	Adsorptive Capacity (%)[b]	Final Rice MC (%)[c]
Intimately Mixed	200	72	26.3 (AB)	11.9 (BC)
Intimately Mixed	500	182	23.1 (C)	12.5 (E)
Intimately Mixed	1000	362	24.4 (BC)	12.4 (DE)
Intimately Mixed Agitated	200	72	27.8 (A)	11.6 (A)
Intimately Mixed Agitated	500	182	25.4 (ABC)	11.6 (AB)
Intimately Mixed Agitated	1000	362	26.4 (AB)	11.6 (A)
Surface Placement	200	72	25.5 (ABC)	12.2 (CD)
Surface Placement	500	182	24.1 (BC)	12.7 (E)
Surface Placement	1000	362	17.8 (D)	14.9 (F)

[b]Each adsorptive capacity observation is an average of two replicates (Fig. 1).
[c]Each final rice-moisture content observation is an average of four measurements. Duplicate oven MC determinations were made for each replicate (Fig. 1).

Table 2. Maximum adsorptive capacities of 1- and 5-g silica gel packets determined by exposing to a moisture-saturated environment for 8 d. Each observation is an average of ten measurements.

Number of Regenerations	Packet Size (g)	Average Mass of Silica Gel Packets (g)		Average Adsorptive Capacity (%)
		Initial Mass (g)	Final Mass (g)	
0	5	4.84	6.66	37.6
0	1	1.16	1.56	35.3
1	5	4.78	6.64	39.0
1	1	1.14	1.56	36.6
2	5	4.78	6.64	39.0
2	1	1.14	1.55	35.7

Table 3. Adsorptive capacities (%) for new, once-, and twice-regenerated desiccant packets from CL730 and Wells cultivars calculated from a linear regression of the final rice moisture content (MC) against assumed adsorptive capacities (15 to 0%) at the final rice MC of 12.5%.

	New desiccant packets[d]	Once-regenerated desiccant packets	Twice-regenerated desiccant packets	New desiccant packets[e]
CL730	28.7	30.2	26.5	26.5
Wells	35.4	34.5	30.9	33.7

[d]Values based on adsorptive capacities ranging from 15 to 30%.
[e]Values based on adsorptive capacities ranging from 15 to 45%.

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Also include one hard copy of each figure camera-ready, printed black on white paper, with the original hard-copy manuscript submission.

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Indicate footnotes using sequential superscript lowercase letters (z, w, x, etc.) Place table footnotes below last horizontal rule of table. Footnotes used to clarify or annotate text should be placed at the bottom of the page in which the reference appears.

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

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The title page should include the following:

- a concise, descriptive title
- authors' first names, middle initials (if any), and last names (faculty sponsor should be listed as a coauthor)
- an abstract
- a footnote identifying each author by classification and major for students; rank and department for faculty and staff
- a footnote identifying faculty sponsor or mentor
- a footnote acknowledging financial support and other assistance. Note support by any companies or parties with a vested interest in the research results.

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

The *Introduction* states the purpose of the study, the hypothesis, and pertinent background information.

The *Materials and Methods* section describes the experimental design, materials used, statistical analysis (required), and any other details needed for another researcher to reproduce the study and to confirm the validity of findings and conclusions.

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 completely supported by your data.

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