Fall 2002

Discovery: The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences - Volume 3 2002

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DISCOVERY

The Student Journal of the Dale Bumpers College of Agricultural, Food and Life Sciences
Vol. 3, Fall 2002

UNIVERSITY OF ARKANSAS
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Cover: Landsat image of Beaver Lake courtesy of Dr. Steve Boss (derived from Arkansas Interactive Mapper, Center for Advanced Spatial Technologies, University of Arkansas; visit http://www.cast.uark.edu/products/MAPPER/)

Letter from the Dean

The vision of the University of Arkansas as a nationally competitive, student-centered research university serving Arkansas and the world is realized in the Discovery journal, which publishes reports on research and creative projects by undergraduate students in the Dale Bumpers College of Agricultural, Food and Life Sciences.
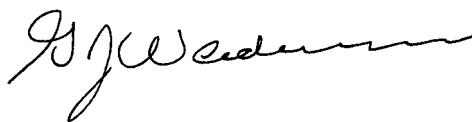
In Bumpers College, "student-centered" means, among other things, abundant opportunities for students to work with faculty mentors on research and creative projects. The College provides research grants specifically for student projects. Most of the articles in this issue are on projects that are part off a U of A Division of Agriculture research program, which may involve a team of faculty scientists, graduate students, and in some cases undergraduate students.

Gregory J. Weidemann

Anyone with an interest in Bumpers College has heard before, and will hear again, about the synergy from the linkage of Division of Agriculture research and extension programs with the academic programs of the College. Our classroom professors are also scientists who conduct Division of Agriculture research on issues that are important to Arkansas and the world. This publication is just one manifestation of that synergy.

These student scientists learn the scientific method by practicing it. They review the literature in their discipline to determine if a void in knowledge or understanding exists that they might help fill. They design experiments to collect objective data, or they establish a protocol for subjective observations that provide a basis for informed analysis. They interpret and explain the significance of their findings and observations.

Many of the student authors are in the Bumpers College Honors Program. They find that engaging in scholarly activities beyond the normal course work helps bring into sharper focus principles they learn in class.

Each of these articles represents a large investment of time and energy by the students and by their faculty mentors. It is time and energy well spent. Our college, our university, our state and our world are well served by these contributions to the various scientific and academic disciplines represented.

Gregory J. Weidemann, Dean
and Associate Vice President for Agriculture

Undergraduate Research Articles

Extraction of silymarin compounds from milk thistle (*Silybum marianum*) seed using hot, liquid water as the solvent

J.F Alvarez Barreto*, D.J Carrier§, and E.C. Clausen†

ABSTRACT

High-value specialty chemicals are usually obtained from natural products by extracting with generally regarded as safe (GRAS) solvents. Because organic solvents are quite often used, high operating and disposal costs occur. When compared to traditional solvents, water is an interesting alternative because of its low operating and disposal costs. Milk thistle contains compounds (taxifolin, silychristin, silydianin, silybinin A, and silybinin B) that display hepatoxic protection properties. This paper examines the batch extraction of silymarin compounds from milk thistle seed meal in 50°C, 70°C, 85°C and 100°C water as a function of time. For taxifolin, silychristin, silybinin A, and silybinin B, extraction with 100°C water resulted in the highest yields. After 210 min of extraction at 100°C, the yield of taxifolin was 1.2 mg/g of seed while the yields of silychristin, silybinin A, and silybinin B were 5.0, 1.8 and 3.3 mg/g of seed, respectively. The ratios of the extracted compounds, and particularly the ratios at long extraction times, showed that the more polar compounds (taxifolin and silychristin) were preferentially extracted at 85°C, while the less polar silybinin was preferentially extracted at 100°C.

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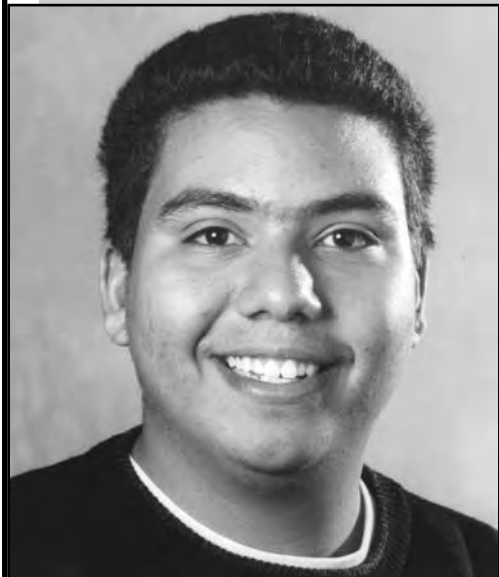
José Alvarez

I was born in Cumaná, Venezuela, in front of a beautiful beach. When 4 years old, I moved to the industrial capital of my country, Valencia. I have always been interested in sciences and graduated from high school in this branch. Then I enrolled in chemical engineering, which really was not my favorite area. One year later, I joined a Science Society for Chemical Engineering Students (SOCITEC), where I contributed to the development of many activities related to the area. Three months later, I was elected as the academic coordinator and had to carry out several projects that literally made me fall in love with my career. These projects were mainly in the area of surfactants and catalysis.

In the year 2000, I had the opportunity to transfer to the University of Arkansas. In this institution I participated in interesting research projects in the area of biotechnology, but I always liked catalysis better. Nevertheless, I really enjoyed my work and learned a lot. I graduated in Spring 2002, and now I will start my PhD program in heterogeneous catalysis at the University of Oklahoma, which is an important step to become a researcher. Later in life, I would like to teach in a university in Venezuela.

INTRODUCTION

Milk thistle (Silybum marianum) is an annual or a biennial plant native to the Mediterranean and North Africa. It grows wild throughout Europe, North Africa, the Americas, and Australia, but can also be cultivated (Hamid et al., 1983). The plants can reach a height of 3.1 m with dark shiny leaves and purple to reddish flowers. Milk thistle has an indeterminate growth habitat, resulting in staggered flowering and maturity (Carrier et al., 2002). The seeds of the plant contain a group of flavanoid compounds commonly named silymarin (Tittle and Wagner, 1978).

The dihydroflavonol taxifolin and the flavanolignans (silybinin, isosilybinin, silydianin, and silychristin) are usually encompassed by the term silymarin (Fig 1). Some studies suggest that silybinin reduces the biliary cholesterol concentration (Duke, 1999). It has also been demonstrated that silybinin is useful in the intervention of hormone refractory human prostate cancer (Zi and Agarawal, 1999). Furthermore, the combination of silybinin and silychristin has been found helpful in decreasing the nephritic effects of chemical-induced injury (Sonnenbichler et al., 1999).

Fig. 1. Structures of silychristin (SCN), silydianin (SDN), silybinin (SBN), taxifolin (TXF) and isosilybinin (ISBN).

The Deutsches Arzneibuch (Benthin et al., 1999) procedure for silymarin extraction is a two-step, process in which seeds are first defatted in a Soxhlet extraction with petrol for 4 hrs, followed by a second Soxhlet extraction with methanol for 5 hrs. Using this procedure, reported silybinin yields were 11 mg/g of seed (Benthin et al., 1999). Milk thistle was also extracted using pressurized liquid extraction techniques, in which 12 mg of silybinin/g of seed were obtained (Benthin et al., 1999). In extracting 0.4 mm milk thistle seed meal in a Soxhlet with petrol for 24 hr, followed by an ethanol Soxhlet for 4 hr, Wallace et al. (2002a) reported a silybinin yield of about 16 mg/g of seed meal. The two-fold difference obtained by Wallace et al. (2002a) over Benthin et al. (1999) may not be significant, since the silybinin content of seed batches varies significantly (Carrier et al., 2002).

Wallace et al. (2002a) reported the analysis of three off-the-shelf milk thistle products, of which only two products contained silymarin compounds. Inconsistency between herbal supplement label and product contents is not uncommon. For example, an analysis of ephedra products (Gurley et al., 2000) showed a broad range of ephedra alkaloid content, pointing most likely to manufacturing variation. The lack of consistency among products can be due in part to the extraction step where the desired molecules diffuse from the bulk herb to a solvent phase, usually ethanol, methanol, acetone, hexane, or petroleum ether. To increase the quality of the products, the extraction step should be well characterized, both in terms of rates and appropriate solvents.

The use of hot liquid water as an extraction solvent has recently caught the attention of some researchers (Basile et al., 1998; Kubátová et al., 2001). Water is very useful in extracting polar compounds and may be useful in extracting polar compounds from plant material without prior defatting. In increasing the water temperature up to its subcritical temperature, a decrease in the dielectric constant is observed. For example, water at 250°C displays a dielectric constant of 27, which is in the realm of that of methanol (33) and ethanol (24). As a result, hot liquid (hot/liquid) water has solubility characteristics, at increased temperature, which are similar to ethanol and methanol. The solubilities of anthracene, pyrene, chrysene, perylene and carbazole (Miller et al., 1998) and of d-limonene, carvone, eugenol, 1,8-cineole and nerol (Miller and Hawthorne, 2000) were determined in 289 K
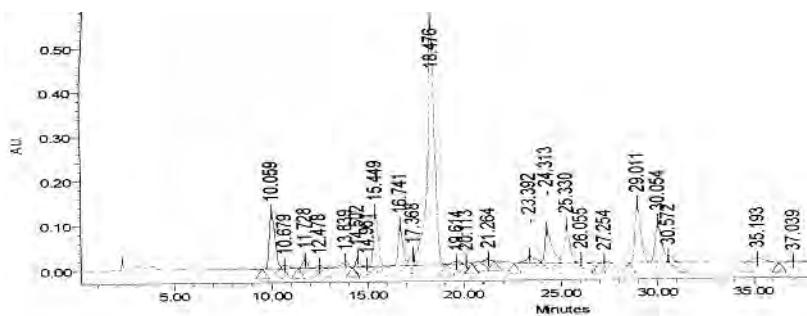
Fig. 2. Typical chromatogram of milk thistle seed extract. Retention times of taxifolin, silychristin, silydianin, silybinin A and silybinin B were 10.59, 18.476, 21.264, 24.313 and 25.330 minutes, respectively. It should be noted that this particular seed lot contained miniscule amounts of silydianin.

Fig. 3. Silybinin B concentration in mg of compound per 100 ml as a function of time at different temperatures. Results show all the batches for all temperatures. The maximum time reported is 240 min; experimentally, infinite times corresponded to 300 min for 100°C experiments, and 1020 min for 50°C, 70°C, and 85°C experiments.

and 498 K (hot/liquid) water, where increases were observed with temperature. Kubátová et al. (2001) showed that the extraction of peppermint compounds using (hot/liquid) water at 175°C required 15 min, as compared to 4 hrs with hydrodistillation. The use of (hot/liquid) water as an extraction solvent shows promise as the search for milder and "greener" solvents is intensifying.

The purpose of this paper is to present results from the extraction of silymarin compounds from milk thistle seeds using (hot/liquid) water as the solvent, a first step in process characterization. Comparisons were made between the compounds extracted as the temperature increased.

MATERIALS AND METHODS

Extraction experiments

Milk thistle seeds were purchased from Frontier Herbs (Norway, Iowa) and ground with a coffee grinder to an average particle size of 0.4 mm. Extraction experiments were conducted at 50°C, 70°C, 85°C, and 100°C, using 2 g of seed (contained in a cheesecloth bag) in 200 mL of deionized water. The leaching at 100°C was carried out in a 500 mL glass round-bottom flask, fitted with a condenser for total reflux. The flask was heated in an electric mantel, and water was used to condense the vapor. The leaching experiments at 50°C, 70°C, and 85°C were carried out in 500 mL bottles in a shaker water bath (Dubnoff Metabolic Shaking Incubator, Precision Scientific, Winchester, Virginia) set at 80 strokes / minute. Although the process conditions were slightly different when operating at or below 100°C, the long diffusion times observed in the experiments helped minimize the small differences in the systems.
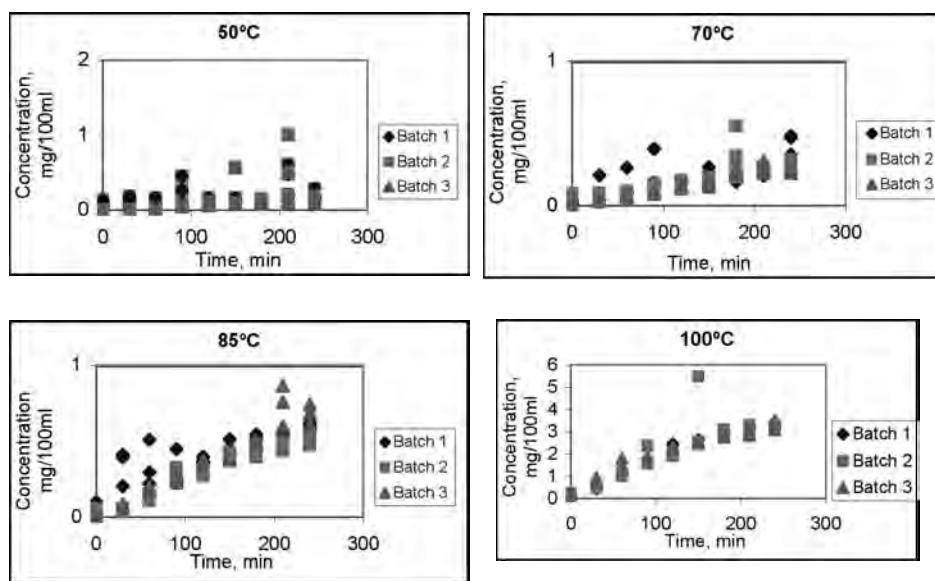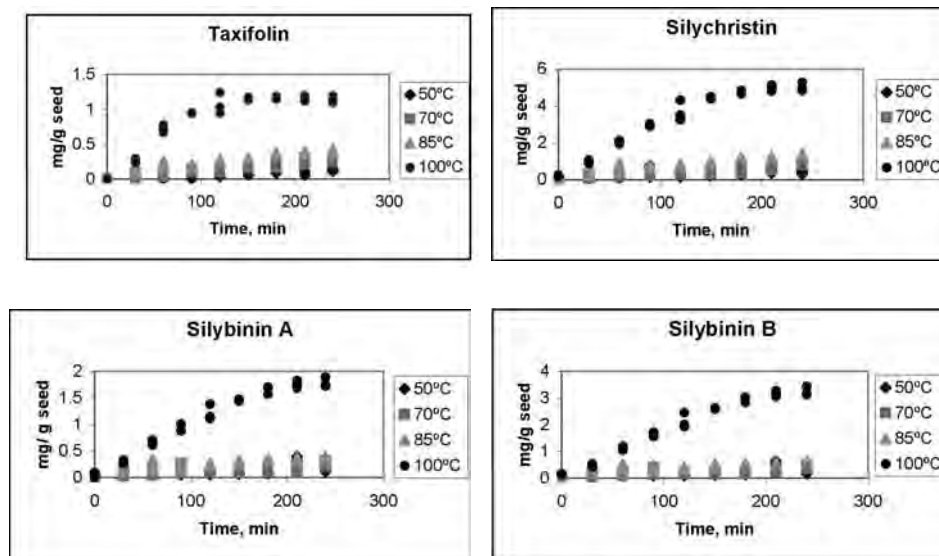

Fig. 4. Concentration in mg of compound per gram of seed as a function of time at different temperatures. results based on the first batch of each temperature. The maximum time reported is 240 min; experimentally, there were infinite times of 300 min for 100°C, and 1205 min for 50°C, 70°C, and 85°C.

Samples of extraction water were taken in triplicate every 30 min, including time 0, using a 1 mL pipette. Time 0 was arbitrarily set as the time when the water started boiling (100°C) or when the temperature of the water in the bottles equilibrated with the set experimental temperature (50°C, 70°C, or 85°C). The aliquots were placed in pre-weighed test tubes and weight determined. Subsequently, the aliquots were evaporated to dryness in a SpeedVac (Savant Instruments, Holbrook, New York). To the dried sample, 1 mL of methanol was added after which they were vortexed and centrifuged (10 g). The supernatant was filtered and analyzed, as described below.

Chemical Analysis

The silymarin concentrations were determined by HPLC using a Waters system (Milford, Massachusetts) composed of an Alliance 2690 separations module and a 996 Photodiode Array, controlled with Millennium32 chromatography software. Separation of the silymarin compounds was obtained using a Symmetry® (Waters, Milford, Massachusetts) C18 pre-column placed in

Table 1: Calculated ratio of compound/Silybinin B as a function of temperature. These ratios were calculated at the last sampling point.

Water Temperature (°C)	Dielectric Constant	Ratio of Compound to Silybinin B (SB)		
	ε	Taxifolin/SB	Silychristin/SB	Silybinin A/SB
50	70	0.916	2.006	0.615
70	64	0.594	1.869	0.639
85	60	0.661	2.237	0.630
100	56	0.352	1.546	0.551

series with a Symmetry® (Waters, Milford, Massachusetts) C18 column (150 mm x 4.6 mm, 5 mm), both at 40°C. A 10 mL sample volume was injected. Solvent A was 20:80 methanol:water while solvent B consisted of 80:20 methanol:water. The gradient program was initiated with 85:15 solvent A:solvent B flowing for 5 min followed by a linear gradient of 45:55 solvent A:solvent B for 15 min. The proportions of 45:55 solvent A:solvent B were then held constant for 20 min and brought back to 85:15 solvent A:solvent B over 10 min. The flow rate was 0.75 mL/min, and the silymarin compounds were monitored at 290 nm. Peak identification was confirmed by mass spectrometry (Pharmalytics, Saskatoon, Saskatchewan, Canada). Calibration curves were prepared with silybinin from Sigma (St. Louis, Missouri), taxifolin from Extrasynthese (Lyon, France), and silychristin and silydianin from PhytoLab (Hamburg, Germany). No standard was available for isosilybinin and thus this compound was excluded from the analysis. The silybinin standard obtained from Sigma contained two distinct peaks, which are further referred to as silybinin A (the first peak) and silybinin B (second peak). A sample chromatogram from the extraction of milk thistle seeds is shown in Fig. 2. The HPLC procedure was previously described by Wallace et al., (2002a).

RESULTS AND DISCUSSION

For all temperatures, three distinct experiments were conducted from which three samples were taken per time point (total of nine samples per time point). Fig. 3 demonstrates the reproducibility of the concentration-time data at each temperature by showing the silybinin B concentration in the extract water with time. The reproducibility of the data improved with increasing temperature as the concentration of the extracted compound increased.

Fig. 4 shows typical results from the extraction of taxifolin, silychristin, silybinin A, and silybinin B presented as the yield of each compound (mg /g of seed) as a function of time and temperature. Each of the extracted compounds showed a consistent pattern of increasing yield with temperature and time. For each of the silymarin compounds, extraction with 100°C water produced the highest yield and concentration of compounds. After 210 min of extraction at 100°C, the yield of taxifolin was 1.2 mg/ g of seed while the yields of silychristin, silybinin A, and silybinin B were 5.0, 1.8 and 3.3 mg per g of seed, respectively. After 300 min of extraction, the yields of taxifolin, silychristin, silybinin A, and silybinin B were 0.92, 4.7, 1.8, and 3.4 mg per gram of

seed, respectively (data not shown). A slight decrease in the yield of taxifolin was observed after 150 min, perhaps indicating the onset of decomposition. Water extraction at 100°C yielded about half of the amount of the silybinins obtained in the two-step Soxhlet extraction (with defatting) performed by Wallace et al. (2002a).

The ratios of the concentrations of taxifolin, silychristin, and silybinin A to the concentration of silybinin B at 85 and 100°C as a function of time are shown in Fig. 5. These temperatures were chosen because the flavanolignan concentrations were not as large at temperatures below 85°C. As is noted in Fig 5 (top left), at 85°C the ratio of taxifolin to silybinin B increased rapidly to 0.7 g/g and then held constant at that level. At 100°C, the
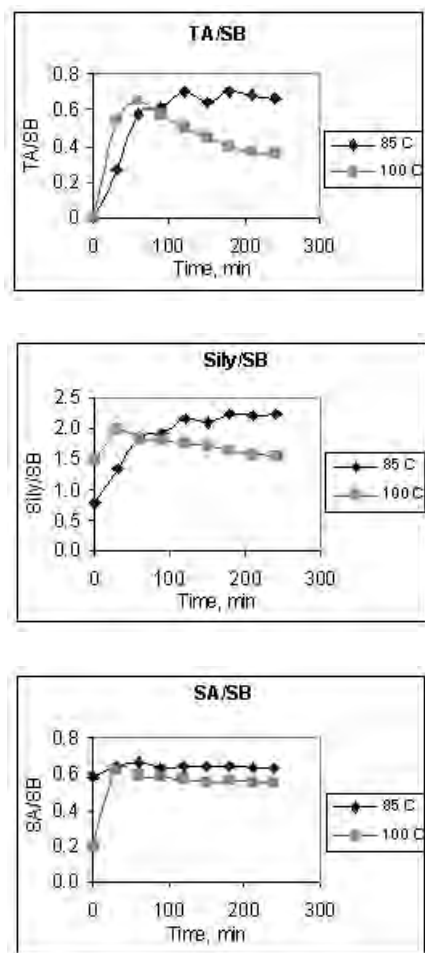

Fig. 5. Compound ratio as a function of sampling points for the 85°C and 100°C experiments. Top (A) taxifolin to silybinin B ratio. Middle (B) silychristin to silybinin B ratio. Bottom (C) silybinin A to silybinin B ratio.

ratio reached a maximum of 0.65 g/g and then gradually fell with time to 0.35 g/g. This reduction in the ratio at 100°C shows that the taxifolin concentration reached its maximum faster than silybinin B. A similar behavior for the ratio of silychristin to silybinin B is noted in Fig 5 (top right). At 85°C, the ratio rapidly rose to just above 2.0 g/g, and then gradually increased before leveling out at 2.2 g/g. At 100°C, the ratio increased to a maximum of 2.0 g/g and then gradually fell to 1.5 g/g. The data of Fig 5 (bottom) show that, excluding an initial sharp increase, the ratio of silybinin A to silybinin B at 85°C was constant at 0.65 g/g. At 100°C, the ratio was constant at about 0.6 g/g again excluding the initial sharp period of increase.

These ratios, and particularly the ratios at long extraction times, show that the more polar compounds (taxifolin and silychristin) are preferentially extracted at 85°C while the less polar compounds (silybinin A, and silybinin B) are preferentially extracted at 100°C (see also the data of Table 1). The data reported by Wallace et al. (2002a) showed that the ratios of taxifolin to silybinin B, silychristin to silybinin B, and silybinin A to silybinin B were 0.02, 0.1, and 0.6, respectively. Thus, the ratios of extraction products using water at 100°C more closely resemble the Soxhlet extraction results than the water extractions at temperatures below 85°C. More dramatic differences in polar and nonpolar compound extraction with water are expected as the temperature of liquid water is further increased, thereby lowering the dielectric constant.

Although the yields of taxifolin, silychristin, silybinin A, and silybinin B using water are half of what is reported in ethanol (Wallace et al., 2002a), this technology shows promise because of the omission of the defatting step. An oil removal step was found necessary in the extraction procedures in acetonitrile proposed by Kahol et al. (2001) and in methanol by Benthin et al. (1999). The work of Wallace et al. (2002b) will examine the extraction of defatted milk thistle seed meal using ethanol, methanol, acetonitrile and acetone as the solvent, establishing a platform on which the present water work will be compared to.

Water is not only an interesting alternative solvent because of its low purchase and disposal costs, it is also highly effective in extracting the silymarin compounds from milk thistle seed. For each of the compound, extraction with 100°C water gave the highest yield and concentration. After 210 min of extraction at 100°C, the yield of taxifolin was 1.2 mg/ g of seed, while the yields of silychristin, silybinin A, and silybinin B were 5.0, 1.8, and 3.3 mg/g of seed, respectively. The ratios of the extracted compounds, and particularly the ratios at long extraction times, showed that the more polar com-pounds (taxifolin and silychristin) were preferentially extracted at 85°C, while the less polar compounds (silybinin A and B) were preferentially extracted at 100°C.

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Identifying safety strategies for on-farm grain bins using risk analysis

Raymond S. Avery*, Dylan P. Carpenter§, and Thomas A. Costello†

ABSTRACT

The potential for grain bin accidents exists each year on Arkansas farms and farms across the nation. The trend toward increasing utilization of on-farm grain drying and storage could lead to an increase in grain bin accidents. The sharp contrast between a safe, efficient operation and one that leads to injury or death can be represented as sets of farmer-decisions and subsequent chance events. A model was constructed to define the risk associated with grain bin entry and in-bin activity so that safety interventions could be identified and implemented to reduce the probability of injury and death. A survey was distributed to Arkansas grain farmers to gather data on the level of safety education, storage techniques, operations management, and other parameters. The data collected from the survey provided quantitative input of many of the model's probability-distribution functions. Using a fault tree (with parallel modes of failure) in conjunction with a Monte Carlo simulation technique, we evaluated six safety intervention strategies and identified the one with the greatest potential for reducing the risk of serous injury or death. As part of senior design in biological engineering, plans are underway to design and test a probe that can locate and break bridged grain (a common risk factor in grain bin management) while working outside the bin on the ground.

* Raymond S. Avery will graduate in May 2003 with a degree in biological engineering.

§ Dylan P. Carpenter will graduate in December 2002 with a degree in biological engineering.

† Thomas A. Costello, faculty sponsor, is an associate professor in the Department of Biological Engineering.

MEET THE STUDENT-AUTHORS
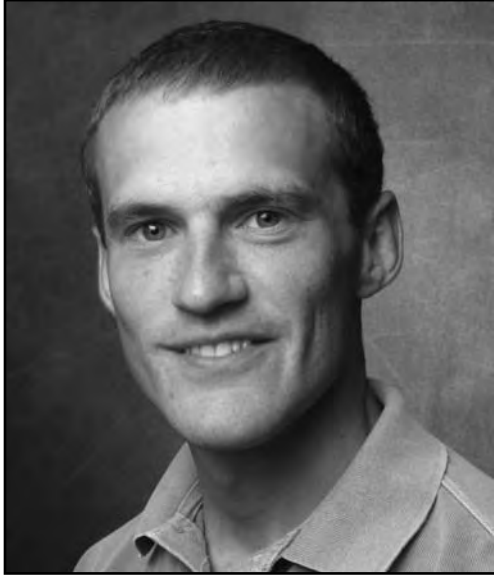
Ray Avery

I am a senior from Paris, Ark., majoring in biological engineering and plan to graduate in May 2003. I was a member of the 3rd place team at the 2001 AGCO national student design competition for our design of aeroponic growth chambers. I am a member of the American Society of Agricultural Engineers (ASAE) and am currently working with researchers to fabricate small mobile wind tunnels for studying ammonia volatilization. I chose this design project because of the long history of safety issues associated with grain bins on small farms.
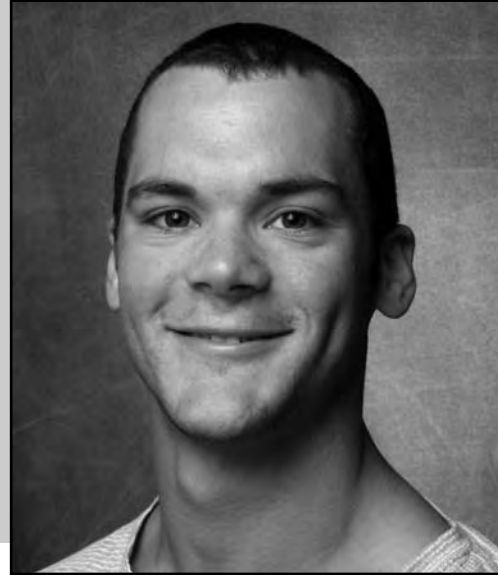
Dylan Carpenter

I was raised in Waldenburg, Ark., and graduated valedictorian from Weiner High School. I worked on the family farm managing crops and live fish. I am now a senior in biological engineering and a recipient of the Chancellor's Scholarship, Xzin McNeal Scholarship, and J.R. Riggs Scholarship. My plans include a future in medicine. I am an ambassador for biological engineering and an active member in ASAE, golden key, and alpha epsilon. Undergraduate research in proteomic analysis of thermozymes has stimulated my interest in the application of genomic and proteomic information to improve human health and well-being.

INTRODUCTION

Commercial grain storage facilities are bound by OSHA regulations, which provide safety standards such as those for confined space entry (OSHA, 1996). However, farms consisting of less than 10 employees are exempt from OSHA guidelines. Because on-farm grain storage is being increasingly utilized, safety concerns are mounting due to common misconceptions about the hazards of grain entrapment and suffocation. Research shows that many operators are unaware of how grain

flows from a bin under different conditions (Loewer and Loewer, 1993). When a metal storage bin is emptied using a bottom-unloading auger, the grain at the top is removed before the grain at the bottom (Loewer and Loewer, 1993). Once grain begins to flow, it expresses the physical properties of a fluid; however, at rest the grains are a complex matrix of individual solid particles. Farmers who fail to understand the nature of flowing grain may unwittingly put themselves or others in dangerous situations. It takes approximately 3 seconds to remove the volume of grain displaced by a 73-kg (160-lb)

person using a 20-cm (8-in) auger in a typical on-farm grain handling system (Kingman et al. 2001). Grain bin operators tend to think that they could free themselves from grain engulfment by their own strength. However, it would be impossible or debilitating to produce and exert the force required to extract a person from total submersion (Schwab, 1994). In order to prevent suffocation due to grain entrapment, the most hazardous conditions and sequences of farmer decisions, actions and outcomes need to be identified and analyzed. With this knowledge, engineering and expert management solutions can be developed.

In the engineering design process, probability uncertainties must be justified through a logically sound method. Risk assessment involves the quantification of potential failure modes in an operation and the failure types, likelihood, and consequences (Wang and Rousch, 2000). Risk assessment and ultimately risk management can be used to optimize the design process by addressing and reducing the amount of uncertainty and potential for catastrophic failure. In order to design grain bin safety devices, we need to properly identify, understand, and quantify the probability of various modes of failure causing grain-bin accidents.

While working inside grain bins, four potential hazards may exist.

1) A significant danger occurs when the farmer-operator goes into a bin while the unloading auger is in operation and grain is flowing. Flowing grain exhibits fluid properties that may engulf a worker before escape is possible. Proper safety measures include locking the auger control in the off position before bin entry. Educational efforts have been targeted at clearly describing this hazard so that farmers will avoid this dangerous situation (Loewer and Loewer 1993).

2) Grain in poor condition (e.g., moist, moldy or decomposing grain) may create a bridge that alters or stops the flow of grain during unloading. The bridge and subsequent partial unloading may create a cavity in the grain mass. If the bridge collapses suddenly, grain will flow to fill the void below. An operator working at the grain mass surface could be rapidly engulfed and covered by an avalanche of grain (Kingman et al. 2001). The intrinsically safe method would be to break the bridge or obstruction without entering the bin (an external method) using a specialized device (such as an Archmaster from Mole-Master Services Co., Marietta, OH) that are used in commercial grain facilities. If bin entrance is necessary, a harness and lifeline should be used with assistants at the bin entrance and on the ground (as required by OSHA for commercial operations, OSHA, 1996). Currently, most farmers do not have access to such safety devices and would probably

not seek and employ additional workers for assistance.

3) Once grain unloading is nearly complete and residual grain is moved using the horizontal sweep auger at the bottom of the bin, vertical crusting of residual grain along the bin wall may become a hazard. Tremendous pressure on the grain during storage and poor physical quality of the grain can produce a wall of crusted grain with a slope much greater than the angle of repose (Loewer and Loewer, 1993). If the farmer attempts to remove the residual crust while working from the bin floor, the vertical wall of grain may collapse and cover the worker (Loewer and Loewer, 1993). Proper grain harvesting and storage techniques (cleaning, drying, and aeration) are needed to prevent crusting. When it does occur, workers should attempt to dislodge the grain by operating from above (using a harness and lifeline).

4) Kingman et al. (2001) showed that nearly 40% of the documented grain suffocations in the U.S. occurred in children under age 15. Therefore, safety education could be used to reduce these accidents by changing the way farm parents supervise their children (i.e., not allowing children to play around grain bins and grain wagons).

The objective of this project was to determine distinct decision progressions and failures that lead to accidents and to identify safety interventions that will have the greatest benefit in terms of reducing the likelihood of serious injury or death associated with on-farm grain storage. The success of any proposed safety intervention will be determined not only by the effectiveness of the design in providing logistical help to the farmer to avoid entrapment and suffocation, but also by the likelihood that the method will be available and implemented. Hence, economics, convenience and education must be considered since safety is essentially a voluntary activity for small operators.

MATERIALS AND METHODS

A hazard analysis was used to define the possible reasons for bin entry that may lead to entrapment. We defined the greatest hazards to be: 1) collapse of a void below bridged grain following flow interruption; 2) engulfment in flowing grain during bin inspection while unloading; 3) children playing in bins; and 4) collapse of vertically crusted grain. A risk assessment was performed to better understand the sequence of events that lead to the 4 hazards listed above. This process explicitly showed how potentially unsafe actions usually avoid injury or death because of a fortuitous (and actually highly likely) sequence of events. However, given a large
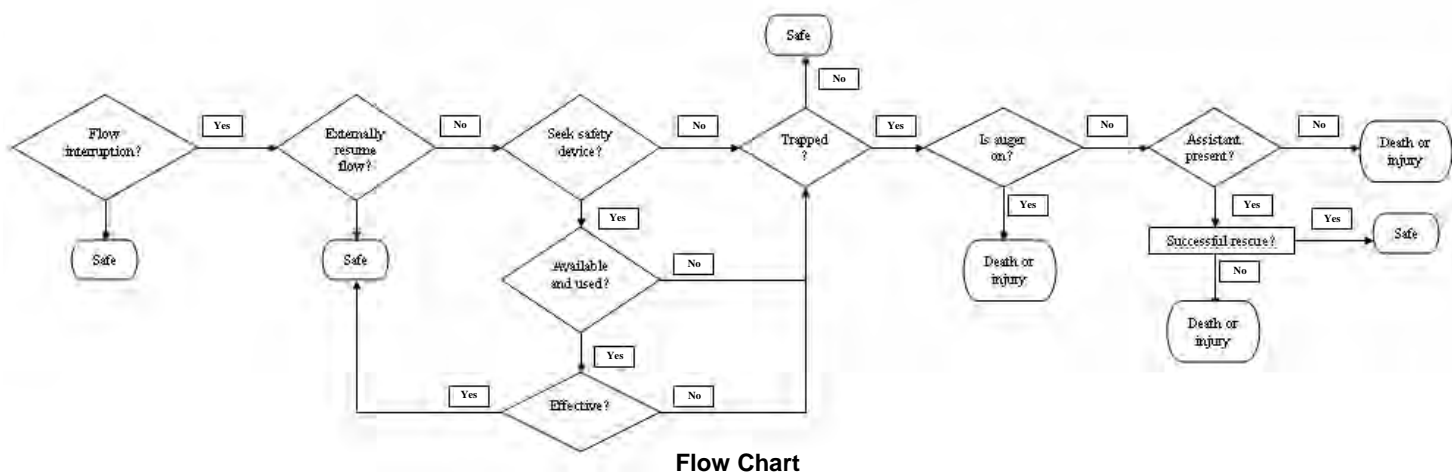
Flow Chart

Fig. 1. Portion of the fault tree used in the risk assessment. An example of a parallel decision-process flow chart due to a flow interruption, which illustrates the farmer's problem-solving process that could result in injury/death or a safe outcome. For example, education could persuade a farmer to seek a safety device that resumes normal flow from outside the bin thereby preventing exposure to hazards directly linked to injury or fatality.

number of grain bin operations, eventually the fatal combination of events will occur. Our hypothesis is that a well-designed safety strategy could precisely place a roadblock that would prevent a fatal chain of events while operating within the farmer's logistical and economic constraints.

A fault tree (Fig. 1) was constructed using Precision Tree™ software (Palisade Corporation, Newfield, NY) to describe the parallel and sequential chance events and decision processes that contribute to injury and death from grain bin operations. The fault tree relies on a parallel system to determine the probability of death from each of the hazard modes. With the parallel system approach, the system fails when all of the components fail in any parallel mode (Haimes, 1989). The probability of death per year per farm was found by estimating the number of times a hazard occurs (the exposure) and by independently examining each mode of failure to estimate the probability of failure.

A survey was composed and distributed through county extension agents to on-farm storage operators in each of the top 10 grain-producing counties in Arkansas. The survey covered relevant information about each farm such as the number of grain bins, cropland area devoted to grain crops, fraction of harvested grain stored on-farm, perceptions of the likelihood of accidents, frequency of routine events, frequency of problem events, participation in education, opinions on factors that cause grain bridging, problem-solving decisions, and attitudes on the use of safety devices.

The survey helped define a base model of the existing hazard exposures and decision-making processes of farmers in the region prior to any intervention. For example, responses to a group of problem-solving questions were used to define a probability distribution for the likelihood of a farmer entering a bin to break a bridge. The probability distribution function was defined by estimating minimum, most likely (mean), and maximum values of a triangle distribution function in @Risk software (Palisade Corp., Newfield, NY). The triangle distribution was simple and robust and allowed the farmers' expert opinions to be directly used as input-probability density functions in the model. A Monte Carlo simulation then randomly chose values from each of the 113 independent model input distributions in the decision tree and performed 100,000 iterations. This simulation technique provided results to quantify the probability of injury or death.

A sensitivity analysis was performed to identify those key parameters which had the greatest impact on the probability of injury or death. Based upon the sensitivity analysis and insight gained through the process of constructing the fault tree, six potential safety interventions were proposed: 1) Educational safety program, 2) External probe bridge breaker, 3) Automated auger locking system, 4) Internal cable bridge breaker, 5) Safety harness and self-locking lifeline, and 6) Combination of (1) and (2). For each intervention (described further in the next section), the distribution functions in the model were modified to represent an estimated change in the

farmer's decisions and actions associated with that intervention. The Monte Carlo simulations for each separate intervention were then compared to the base model to compute the estimated mortality reduction (Table 1).

RESULTS AND DISCUSSION

Of the approximately 130 surveys sent, 69 farmers responded (53% response rate). The average farm produced 930, 1400 and 420 acres of rice, soybeans and wheat, respectively. On-farm grain storage (average of 10.4 bins per farm) was utilized for 73, 24, and 14% of the harvested rice, soybeans and wheat, respectively. The farmers surveyed believed that collapsing bridges (64% of respondents) and auger engulfments (62% of respondents) were two causes of accidents most likely to occur (Fig. 2); however, survey results showed that these accidents are considered rare. To avoid these rare catastrophes at least 50% of the respondents stated that they would have a helper present, turn off the equipment, and try to break a bridge from outside the bin before entering the bin (Fig. 3). Farmer's also expressed in the survey (data not shown) a willingness to participate in educational programs, which indicates potential for safety program development. Survey results suggested that an external bridge breaking device coupled with safety education might be the optimal safety intervention.

The risk assessment model (coupled with realistic inputs based on the survey) is an engineering tool that was used to optimize the final design solution. The survey facilitated construction of a model that represented the personal knowledge, practices, and decision-making processes of farmers in the grain-producing region. This is critical since these factors vary geographically due to differences in climate, soils, crop selection, farm practices, education, and culture. For example, national statistics indicated that the majority of grain suffocations and injuries occur with children; however, our survey results suggested that the majority of farmers in the Arkansas sample believed that children are less endangered. Approximately 70% of responses agreed with a statement that children "never" climb on, look at or play in or near the bin. The deviation between the survey results and the actual statistics represents a need for education and precise tracking of farm accidents.

From the base model Monte Carlo simulation, the mean predicted value of deaths resulting from grain bin entry in Arkansas was 0.92 per year (Table 1). The actual number of deaths related to grain bin entry in Arkansas is difficult to determine, but is estimated to be one death every two to three years (Huitink, 2002). There is uncertainty associated with the input probability distribution functions (particularly those that define numbers of entrapments and deaths associated with

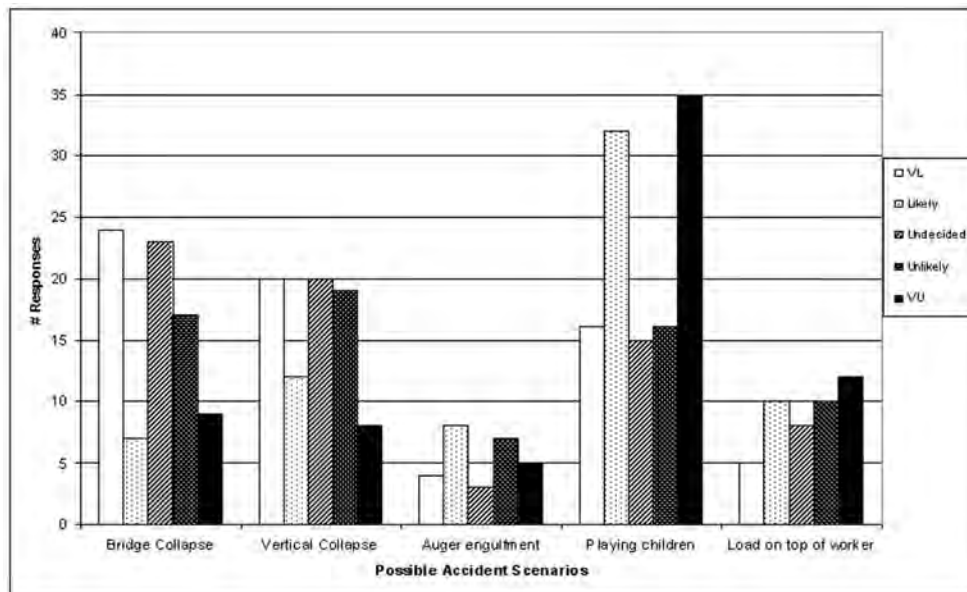**Fig. 2.** Likelihood of accidents. Survey responses for farmers' estimates of the likelihood that a person could be trapped, injured or killed by an accident involving (1) collapse of bridged grain, (2) collapse of vertically crusted grain, (3) engulfment in flowing grain while the unloading auger is running, (4) children that entered bins to play, (5) grain loaded on top of a person inside the bin.
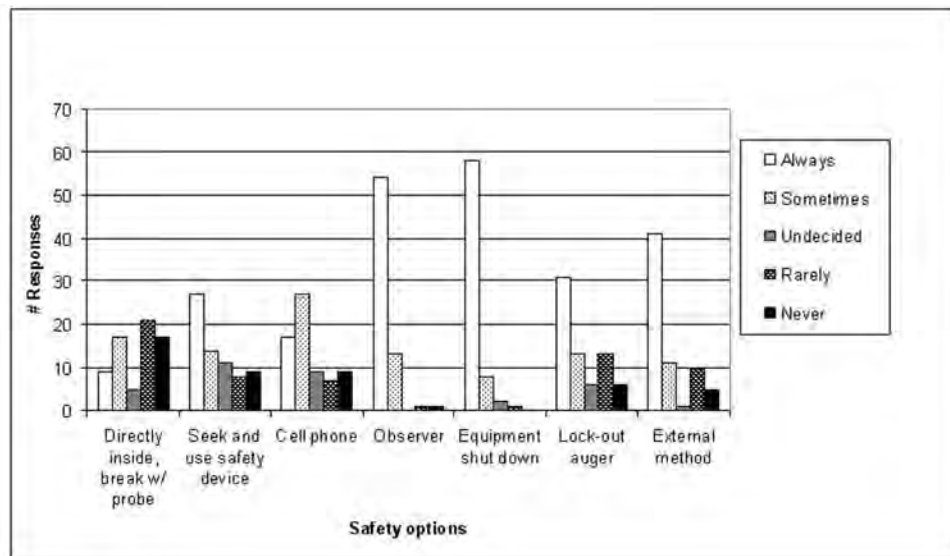
Fig. 3. Problem-solving decisions. Survey responses for farmer's actions when grain quits flowing during unloading and bridging is suspected.

Table 1. Results of safety intervention analysis in on-farm grain storage showing reduction in predicted mortality associated with six safety interventions.

Intervention	Hazard	Affected nodes	Triangle distribution parameters (max, mean, min)		Mortality (deaths per year in Arkansas)		
			base model	intervention model	Base	Intervention	Reduction
Education	Bridge	Seek safety device	(0.068, 0.41, 0.99)	(0.068, 0.93, 0.99)	0.92	0.89	3.3%
		Auger off	(0.068, 0.89, 0.99)	(0.062, 0.88,0 .99)			
		Observer	(.068, .88, .99)	(.062, .88, .99)			
	Children	Child attracted	(0.15,0.25,0.3)	(0.1,0.175,0.25)			
		Child enter bin	(0.05,0.1,0.15)	(0,0.05,0.1)			
	Vertical crust	Safe distance	(0.2,0.6,0.7)	(.02,0.3,0.4)			
External probe bridge breaker	Bridge	Break from outside (large cavity)	(0.1,0.25,0.35)	(0.8,0.9,1)	0.92	0.37	60%
		Break from outside (small cavity)	(0.65,0.75,0.85)	(0.8,0.9,1)			
		Device available and used	(0,0.05,0.1)	(0.2,0.2045,0.4)			
Auger-Lock	All	Auger off	(0,0.88, 0.99)	(0.93, 0.96, 0.99)	0.92	0.92	0.94%
	All	Device available and used	(0,0.05,0.1)	(0.1,0.28,0.38)			
Internal cable bridge breaker	Bridge	Device available and used	(0,0.05,0.1)	(0,0.06,0.1)	0.92	0.87	6.1%
	Bridge	Break from outside	(0.1,0.25,0.35)	(0.2,0.3,0.4)			
Harness/lifeline	Bridge	Trapped (break bridge)	(0,0,0.0002,0.005)	(0,0,0.0002,0.0005)	0.92	0.78	15%
		Trapped (no break)	(0,0,0.0018,0.005)	(0,0,0.00018,0.0005)			
	Vertical crust (tall/short)	Trapped (break safe distance, on grain)	(0,1E-5,5E-5)	(0,1E-6,5E-6)			
		Trapped (danger distance, on grain)	(7.5E-2,8.5E-2,9.5E-2)	(7.5E-3,8.5E-3,9.5E-3)			
	Inspection	Trapped (structure)	(4E-4,5E-4,1.2E-3)	(4E-5,5E-5,1.2E-4)			
		Trapped (sampling)	(4E-4,5E-4,1.2E-3)	(4E-5,5E-5,1.2E-4)			
	All	Device available and used	(0,0.05,0.1)	(0.1,0.1925,0.3)			
Education & external	Combined				0.92	0.35	63%

each specific exposure) and biased survey results. Research is needed to understand and quantify entrapment and mortality probabilities. A wider survey base along with better regional statistics should be sought to increase the model's precision.

An optimized solution was found by comparing the mortality reduction of each intervention.

(1) Education reduced the mortality rate by only 3%. This may be an underestimate of the value of education. The base model was calibrated to farmer's survey responses, in which they reported that they utilize good safety practices, however, we suspect that in reality, short cuts are often taken. Education could help farmers recognize a potentially lethal situation and take safety precautions.

(2) The external-pole bridge breaker (EPBB), was envisioned as a device that would allow a farmer to break bridged grain from an external ground position. It reduced mortality rate by 60% because it affected the fault tree in one of the most sensitive nodes (and required no bin entry).

(3) Automatic auger-lock (to prevent auger operation while someone is in the bin) showed only a 1% reduction in mortality rate because getting trapped during routine unloading represented a small number of predicted fatalities.

(4) The internal cable bridge breaker was envisioned as a cable/winch system installed inside the bin (before loading) which could be retracted and pulled up through the grain surface and possible bridges if flow interruption occurred. It reduced mortality by only 6% because it was considered less effective than the EPBB (when it failed the farmer would resort to in-bin methods).

(5) The harness/lifeline lowered the probability of becoming trapped and resulted in a 15% mortality reduction. We predicted that it would be used less often than the EPBB due to cost and logistical factors. The harness/lifeline also involved bin entry.

(6) A combination of the external-pole bridge breaker and education resulted in a 63% mortality reduction. This was a slight improvement over EPBB alone because education increased the likelihood that a safety device would be used.

The combination of EPBB and education was identified as a preliminary design concept for an engineering solution to the grain bin safety problem. A prototype of the external bridge-breaking probe, with a vibrating head and inflatable bladder that will allow a farmer to reach and break bridged grain from an external location has been constructed and tested as part of our senior design project in biological engineering. Development of suggestions for safety education in Arkansas is under consideration as well.

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Preliminary results from a survey on the prevalence of parasitic helminths and protozoa in raccoons, opossums, and skunks, with special reference to *Baylisascaris* spp.

Michelle Belviy*, T.A. Yazwinski§, C.A. Tucker†,
and Jennifer Robins

ABSTRACT

Raccoons, skunks, and opossums (N=57, 60, and 60, respectively) were necropsied for parasite detection and identification from September, 2001 until April, 2002. Qualitative coprological exams and adult Baylisascaris collections have been completed. Fecal stages and/or types found were Baylisascaris and Strongyloides-type (skunks and raccoons); Capillaria and Trichostrongyle-type (raccoons and opossums); Acanthacephalan and ascarid type (opossums only); free larvae (skunks only); and coccidial (protozoan) oocysts (all three host species). Adult Baylisascaris were recovered from 33.3% of the raccoons and 58.3% of the skunks. Data collection relative to this survey, which is still ongoing, includes the determination of Sarcocystis prevalence in excised skunk and raccoon muscle as well as prevalence and magnitude of the numerous enteric helminths recovered from the three host animals.

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INTRODUCTION

Many pathogenic organisms are reservoired in wild (sylvatic) animals, which cause disease in domesticated animals as well as in humans (latter diseases referred to as zoonoses). *Sarcocystis neurona* is a protozoan parasite of the opossum's intestinal tract, but if the parasite is ingested by a horse, central nervous system infection and dysfunction follow, culminating in a disease called equine protozoal myeloencephalitis (EPM) (NAHMS, 2001). All life cycles of *S. neurona* have not been totally defined, especially in regard to the paratenic hosts that both reservoir and distribute this protozoan parasite (Dubey et al., 2001). Exposure of horses to the pathogen is common, with sero-positive rates of 50% or higher (Blythe et al., 1997). Fortunately, only a small percentage of exposed horses develop EPM, with early detection and treatment proving very successful (Fenger, 1996).

Results from the current survey will be valuable in assessing the extent of pathogen availability in our area, a factor directly related to the health threat that exists for horses.

The other parasites targeted in the current survey are *Baylisascaris procyonis* and *B. columnaris* of the raccoon and striped skunk, respectively. These nematodes are entirely enteric in the above hosts, but assume aberrant migrations (visceral, ocular, and neural larval migrans) in paratenic hosts including humans (Kazacos, 1986). These migrations are poorly diagnosed, and therapy is usually not obviated until central nervous system involvement is evident (Kazacos, 1982). Knowledge of the prevalence of these nematodes is therefore very important from the standpoint of human health concerns, especially in relation to those individuals who utilize the more natural parts of our "Natural State".

MEET THE STUDENT-AUTHOR

Michelle Belviy

I graduated in 1999 from Mount St. Mary Academy in Little Rock. I am a junior honors student majoring in animal science with an added pre-vet curriculum. I have received several scholarships and honors over the years including the Target All-Around Scholarship, Pre-Vet Club Scholarship, Arena Seat Scholarship, Meat Sciences Scholarship, University Housing Resident Assistant Scholarship, First Place-Gamma Sigma Delta Undergraduate Research competition, Dale Bumpers Undergraduate Research Grant, as well as several certificates for recognition of academic achievement.

During the summer of 2000, I interned with a veterinarian in Arizona and observed first-hand the procedures of veterinary medicine in the feed lot industry. I plan to graduate in May of 2003 with a B.S. degree in animal science and continue my education in veterinary school.

I am active in and have been an officer for several clubs on campus including Pre-Vet Club, Alpha Zeta, Sigma Alpha, and Student Health Advisory Committee. I am also a member of the National Society for Collegiate Scholars, Gamma Beta Phi, and Golden Key. Currently, I am a resident assistant for the medical sciences floor in Pomfret Hall.

I came upon this field of research because of my fascination with parasitology. Parasites are a major factor in animal health, a field obviously very important to me. This study has helped me to expand my knowledge of animals and their parasites. The information I have gained by studying the symbiotic relationship between animals and helminths, identifying helminths, and learning time management to accomplish all of this, will be an excellent resource for me in veterinary school and beyond.

I would like to thank Dr. Yazwinski, Dr. Chris Tucker, and Jennifer Robins for all the guidance, time, and labor they have contributed to this project.

MATERIALS AND METHODS

Raccoons, opossums, and skunks were obtained starting in September of 2001 and collection continues at this time (May, 2002). The primary source of specimens was recent intact road-kill (< 1 day from time of demise). In addition, live-trapped animals humanely euthanized via gun-shot were also used. These latter animals were obtained under Arkansas Game and Fish Commission permit (License No. 197009 222001 112347). An adequate sample size of 60 animals per species was set as a target, which has been reached for skunks and opossums but not for raccoons (N=57).

All animals were necropsied according to standard protocol as provided below:

(1) Haircoat and skin inspected for external parasites or lesions (i.e. mange).

(2) Abdominal and thoracic cavities opened ventrally along the entire midline.

(3) Intestinal tract from stomach to rectum removed (fecal sample obtained at this time for qualitative parasite egg detection via saturated $MgSO_4$ flotation followed by microscopic examination).

(4) Intestinal tract opened lengthwise and all contents washed over a #120 mesh sieve.

(5) Sieve residue placed in formalin until subsequent inspection, in total, at 10-40x for parasite collection, identification, and counting.

In addition to the above, sections of muscle were obtained from all skunks and the majority of the raccoons for shipment to Dr. Ellis Greiner (University of Florida School of Veterinary Medicine) for research as to the content and identity of encysted protozoan parasites (e.g. S. neurona).

Microscopy was conducted with a stereoscopic microscope for magnifications < 40 X and a compound microscope for magnifications > 40X. Small helminths (<2 CM) were cleared in lactophenol for 24 hrs. prior to inspection and/or photography. In regard to photography, egg and helminth images were received through a color video camera and digitized to a computer for recording.

RESULTS AND DISCUSSION

At the time of this writing, data collection is still in progress. Results from completed qualitative coprological examinations as well as the isolation, identification, and counting of adult B. procyonis and B. columnaris can be reported at this time.

The prevalence rates of the different types of fecal stages detected during the coprological exams are given in Table 1. Pictures of the various stages are given in Fig. 1. Strongyloides-type eggs were the most frequently found in skunk samples—an egg characterized by the inclusion of a motile larva. Additionally, 68% of the skunk samples were positive for coccidial (protozoan) oocysts and 32% were positive for B. columnaris eggs. For raccoon samples, Trichostrongyle-type eggs were the most common, followed in frequency by coccidial oocysts and B. procyonis eggs. The most prevalent egg type found in opossum samples was acanthacephalan ("thorny-head"), a class of parasite only seen in the opossums obtained in the survey.

The opossum is the primary if not sole definitive host for S. neurona. In our survey, only 9.7% of the opossums were passing coccidial oocysts. In addition to a low frequency of oocyst shedding, the oocysts passed by the opossums in the survey did not appear (description and size) to be S. neurona (Cheadle et al., 2001). This apparent lack of S. neurona detection in the surveyed opossums might be due to laboratory technique and chemicals, factors extremely critical for the successful isolation of S. neurona stages (E. Greiner, personal communication). Therefore, documentation of S. neurona presence in Arkansas will hopefully be provided by the histological and molecular (DNA) work done at the University of Florida School of Veterinary Medicine from the raccoon and skunk muscle samples as obtained in this survey.

Characterization of the adult Baylisascaris burdens found in the surveyed raccoons and skunks is given in Table 2. Pictures of these nematodes are presented in Fig. 2. Adult forms of these parasites were found in 33.3% and 58.3% of the raccoons and skunks, respectively. Rates of patent infections (positive for fecal egg counts) were slightly lower due most likely to worm maturity. Overall rates of infection by these nematodes will undoubtedly be higher, once all smaller parasite forms are identified and counted.

As can be seen from the above data, Baylisascaris is of high prevalence in our area. Numerous reports document the adverse effects of this parasite when it is accidentally ingested by humans and migrates to the eye (ocular larval migrans) or brain (neural larval migrans) (Kazacos, 1986; Kazacos, 1997; Kazacos and Boyce, 1989). In addition, non-human vertebrates serving as paratenic hosts also suffer from the nematode's larval migration (Clark et al., 1969; Kazacos, 1981; Stringfield and Sedgwick, 1997). Therefore, without question, this parasite is ubiquitous and poses considerable threat to human as well as animal health and should be scrutinized along with the more notorious sylvatic, zoonotic parasites such as Giardia, Toxoplasma, Echinococcus, and Trichinella.

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Table 1. Prevalence of progeny shedding by type of fecal stage and host.

Characterization of fecal stage/type	Percentage of animals positive by host		
	Skunk	Raccoon	Opossum
Baylisascaris	32.0	25.6	0.0
Strongyloides - type	70.0	7.7	0.0
Trichostrongyle - type	0.0	84.6	41.9
Capillaria	0.0	28.2	32.3
Acanthacephalan	0.0	0.0	87.1
Ascarid - type	0.0	0.0	16.1
Free larvae	22.0	0.0	0.0
Protozoan oocyst	68.0	74.4	9.7

Table 2. Comparative characterization of adult *Baylisascaris* burdens as found in this study.

Item	Host (Nematode)	
	Raccoon (*B. procyonis*)	Skunk (*B. columnaris*)
Arithmetic mean for adult forms in positive animals	13.7	5.0
Range in number of adult forms in positive animals	1-40	1-19
% of animals infected with adult forms	33.3	58.3
% of animals with positive fecal egg counts (% patent)	25.6	32.0

Fig. 1a. Parasitic eggs from opossum, fecal flotation.
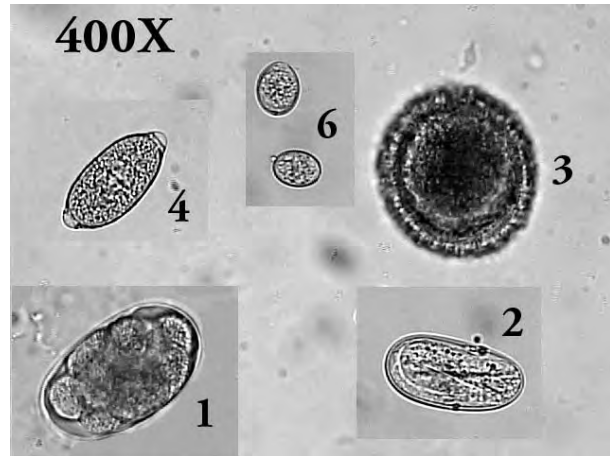

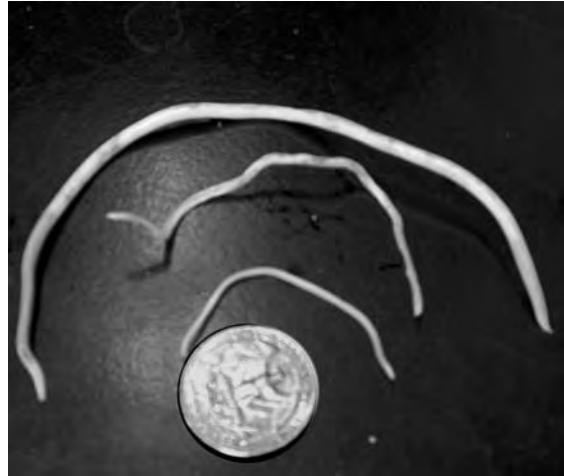**Fig. 1b.** Parasitic eggs from raccoon, fecal flotation.

Fig. 1c. Parasitic eggs from skunk, fecal flotation.

Egg/Oocyst Type
1. *Trichostrongylid*
2. *Strongyloides*
3. *Baylisascaris*
4. *Capillaria*
5. *Acanthocephalan*
6. Protozoan oocyst

Baylisascaris procyonis

Baylisascaris columnaris

Fig. 2. Typical *Baylisascaris* specimens recovered and
counted as adults

Land-use effects on soil-water retention characteristics

Naomi C. Colton* and Kristofor R. Brye§

ABSTRACT

Tillage can negatively affect soil physical properties such as bulk density, organic matter content, and soil hydraulic properties, which in turn affect how plants grow. The objective of this study was to evaluate water retention characteristics of a Jay silt loam soil under cultivated agriculture and native tallgrass prairie in northwest Arkansas. Air-dry soil samples collected from 0-10 cm depth were re-wet with varying amounts of distilled water to create a range of water contents. After overnight equilibration, the water potential was measured on the re-wet soil samples using a dewpoint potentiameter. The relationship between water potential (Ψ) and water content (θv) for the cultivated agricultural and undisturbed prairie soil was modeled using the equation $\Psi = a\theta v^{-b}$, where a and b are coefficients determined from fitting the data and represent the water retention characteristics for the soil of the two different land uses. The a and b coefficients did not differ significantly due to land use. Therefore, the results of this study did not support our hypothesis that agricultural land use significantly affects water retention characteristics. However, increasing the number of soil samples in which the water potential was measured could have sufficiently decreased the variability in the a and b coefficients so that significant differences in water retention characteristics as a result of land use could have been demonstrated.

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INTRODUCTION

Disturbing the soil with tillage can alter soil physical properties. Tillage influences the soil organic matter content and the soil's ability to retain and supply water to plants. Organic matter helps hold sand, silt, and clay particles together to form soil aggregates, which promote good soil structure. Organic matter also increases the soil's capacity to hold water. Consequently structure and organic matter, which are both influenced by tillage, affect water retention in soil.

Tillage also affects soil bulk density. Bulk density is the mass of dry soil per unit volume, which consists of both solids and void space (i.e., pores). Soil with a large volume of void space compared to the volume of solids has a lower bulk density, whereas a typical bulk density for well-structured soil is 1.3 Mg/m^3.

A soil with good structure has both macro- and micropores. Macropores allow water to readily infiltrate the soil. Micropores retain the water so that it doesn't flow through the soil profile too quickly; consequently the water is held for plants to extract. Undisturbed soils that are well structured, such as prairie soils, typically have a greater volume of pore space than cultivated soils because cultivation has disturbed the natural structure

and in some cases caused soil compaction.

Prairie soils that have not been affected by agricultural practices also typically have higher organic matter content than cultivated soils. Since prairie soils are high in organic matter, they also tend to have better structure and water retention characteristics than cultivated soils. In a study conducted by Scott et al. (1983), virgin Dubbs and Sharkey soils from eastern Arkansas were compared to soil of the same series that had been cultivated. Results showed that the virgin soils contained higher amounts of organic matter and retained more water, but had lower bulk densities than the cultivated soils (Scott et al., 1983).

The objective of this study was to compare water retention characteristics of a cultivated and undisturbed Jay silt loam soil in northwest Arkansas. We hypothesized that, similar to the findings in eastern Arkansas, land use significantly affects water retention characteristics.

MATERIALS AND METHODS

Site Description

The study site was located on a 24.3-ha tract of land in Benton County, Arkansas, approximately 4.8 km north of Siloam Springs. This tract of land, known as the Chesney Prairie, was acquired by the Arkansas Natural

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MEET THE STUDENT-AUTHOR

Naomi Colton

I am from Rogers, Ark., where I graduated from Rogers High School. I am a senior majoring in environmental, soil and water science in the Department of Crop, Soil, and Environmental Sciences.

During Summer 2001, I began working for Dr. Brye and got involved in some of his work relating to soil physics and soil-quality assessments of disturbed (i.e., cultivated agriculture) and undisturbed (i.e., native prairie) soils. He took some of the work I was doing and turned it into a research project for me related to land use and water-retention characteristics. This opportunity has given me experience in knowing what goes into conducting a scientific experiment and how to write a scientific paper. The experience will be valuable for me no matter what I do.

I was an Earth Team volunteer during Spring 2002 for the Natural Resource Conservation Service (NRCS) in Washington County, AR. During Summer 2002, I was a student trainee with the NRCS, where I helped conduct and learned what goes into preparing a soil survey.

My future after graduation is undecided at this time. However, I would like to either continue working for the NRCS or attend graduate school to pursue a Master's degree in environmental science.

Heritage Commission (ANHC) in 2000. According to the ANHC, the Chesney Prairie is one of very few prairie remnants in the Arkansas portion of the Springfield Plateau (ANHC, 2001). Within the Chesney Prairie Natural Area, a unique combination of undisturbed prairie and cultivated agricultural land use exists adjacent to each other on the same soil (Fig.1). These two land uses reside on a Jay silt loam soil (fine-silty, mixed, thermic, mollic fragiudalf), which typically exists on the broad uplands of northwest Arkansas and is moderately well drained (Phillips and Harper, 1977).

The topography of the study area is gently rolling with the slope ranging from 1 to 2%. Prairie vegetation at the site includes native grasses such as big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), indiangrass (*Sorghastrum nutans*), switchgrass (*Panicum virgatum*), prairie cordgrass (*Spartina pectinata*), gayfeather (*Liatris pycnostachya*), and numerous other forbs and perennials (ANHC, 2001). The texture of the soil surface is silt loam and the upper part of the subsoil is silty clay loam. The Jay soil series is typically used for pasture and meadow in Northwest Arkansas. However, the cultivated portion of the Chesney Prairie had been typically planted to soybeans (*Glycine max*) in the past (ANHC, 2001). Cultivation was ceased at the site in 2000.

Field Sampling

A 60-m transect was established in the prairie and cultivated agricultural field. Two soil cores, 4.7 cm in diameter, were collected using a slide hammer from the 0 to12 cm depth at five points spaced 15 m apart along the transects. The samples were used for bulk density determination, particle-size analysis, and determination of water retention characteristics.

Laboratory Procedures

One of the two soil cores collected at each of the five points along the transects was air dried for 48 hrs, ground, and sieved through a 2-mm mesh screen. Three of five air-dried soil samples were used to determine water retention characteristics. Nine 5 ± 0.1 gram samples of air-dried soil were weighed out into small cups. Varying amounts of distilled water (i.e., 2, 4, 6, 10, 12, 15, 20, 30, and 40 drops) were added to the cups and the wet soil was mixed thoroughly. The cups were covered and allowed to equilibrate overnight. The following day the water potential of the soil in each cup was measured with a dewpoint potentiameter (Model WP4, Decagon Devices, Inc., Pullman, Wash.). The dewpoint potentiameter measures the water vapor pressure of the air in the sample chamber after the air in the sample chamber has equilibrated with the liquid water in the soil sample. After measuring the water potential, the gravimetric water content of the soil in each cup was determined by drying at 70°C for approximately 10 to 12 hrs.

The second of the two soil cores collected at each of the five points along the transects was weighed, oven dried at 70°C for 48 hrs, and reweighed for bulk density determination. Bulk density (ρb) was calculated by the following equation:

ρb= (mass of wet soil – mass of dry soil)/sample volume [1]

where the sample volume was 208 cm³. Once oven dried, soil samples were ground and sieved through a 2-mm mesh screen. Particle-size analysis was determined on a 40-g subsample of oven-dry soil from each of the five points along the transect by a standard hydrometer method (Arshad et al., 1996).

Statistical Analysis

A one-way analysis of variance (ANOVA) was performed to determine the effect of land use on bulk density and the percentages of sand, silt, and clay (Minitab, 1997). Measured water potentials for each replicate soil sample (n = 3) were plotted against the corresponding volumetric water content, which was calculated by multiplying the gravimetric water content by the soil's bulk density. The equation $\Psi = a\theta v^{-b}$ was fit to the resulting curves using a spreadsheet, where Ψ is the water potential (-MPa); θv is the volumetric water content; and a and b are coefficients determined from fitting the data and represent water retention characteristics of the soil. An ANOVA was also performed to determine the effect of land use on water retention characteristics (i.e., the a and b parameters). Data are reported as mean values with statistical significance among means determined by P< 0.05.

RESULTS AND DISCUSSION

Particle-Size Analysis

Mean percent of sand, silt, and clay were 21.8, 68.6, and 9.7% respectively (Table 1), in the undisturbed prairie. In the cultivated agricultural soil, mean percentages of sand, silt, and clay were 23.5, 67.0, and 9.5% respectively (Table 1). Particle-size analysis demonstrated that the percentages of sand, silt, and clay in the top of the undisturbed prairie and cultivated agricultural field did not differ significantly (P<0.05) (Table 1).

Therefore both soils do indeed have the same soil texture (i.e., silt loam), which is congruent with how the soil in the area was originally mapped. Since the textures are similar, this indicates that the soils being compared are relatively the same and the results hereafter will be a comparison of two like soils.

Bulk Density

Bulk density in the top 12 cm averaged 1.12 and 1.30 g/cm³ in the native prairie and cultivated agricultural

A

B

Fig. 1. Native tallgrass prairie (A) and previously cultivated agricultural (B) land uses at the Chesney Prairie Natural Area near Siloam Springs in Benton County, Ark.

field, respectively (Table 1). The bulk density of the prairie soil was significantly lower (P<0.05) than that of the cultivated agricultural soil (Table 1). This difference indicates that the prairie soil has a greater volume of pore space than the cultivated soil. The greater volume of pore space in the prairie allows water to infiltrate through the soil and be retained more readily than in the cultivated soil. These results for disturbed and undisturbed soils in northwest Arkansas are similar to the finding of Scott et al. (1983) for a similar setting of adjacent disturbed and undisturbed soils in eastern Arkansas.

Water Retention Characteristics

The soil-water potential increased and leveled off as water content increased in the native prairie and cultivated agricultural soil (Fig. 2). However, water retention characteristics (i.e., the modeled a and b coefficient of the equation $\Psi = a\theta v^{-b}$), as determined using soil-wetting curves, did not differ significantly by land use (Table 2). The a coefficient did not differ significantly (P<0.05) among land uses. Similarly, the b coefficient did not differ significantly (P<0.05) among land uses. Therefore, the results of this study, acquired using soil-wetting curves, did not support the hypothesis that land use significantly affects water retention characteristics.

Several reasons may exist to explain these results. Along each transect, five soil samples were collected. However, only three of the five soil samples collected were used to determine water retention characteristics. Had all five soil samples been used to determine water retention characteristics, the variability associated with the mean values of the a and b coefficients would most likely have decreased, which may have resulted in significant differences among mean values for the a and b coefficients. In addition, the hypothesis that land use affects water retention characteristics was based on results from Scott et al. (1983), in which water retention characteristics were determined using soil-drying curves

rather than soil-wetting curves, which were used in this experiment.

In the Scott et al. (1983) study, after obtaining an intact soil core, the soil was saturated, placed in a chamber, and pressurized at various levels to dry the soil core. The intact soil core was neither air dried nor ground and sieved. Therefore, the original structure was left undisturbed. In contrast, the soil samples collected in this study were air dried, ground, and sieved. Altering the original structure of the soil by air drying, grinding, and sieving affected the outcome of this study so that we were unable to demonstrate significant differences in water retention characteristics due to land use.

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Table 1. Summary of the effects of land use (i.e., undisturbed prairie versus cultivated agriculture) on soil particle size and bulk density.

Land use	n	Sand	Silt	Clay	Bulk density
			%		g/cm³
Cultivated agriculture	5	23.5a[z]	67.0a	9.5a	1.30a
Native prairie	5	21.8a	68.6a	9.7a	1.12b

[z] Different letters after mean values represent significant differences (P< 0.05).

Table 2. Summary of the effects of land use (i.e., undisturbed prairie versus cultivated agriculture) on mean water retention characteristics (i.e., the a and b parameters of the model $\Psi = a\theta v^{-b}$).

Land use	n	a coefficient	b coefficient
Cultivated agriculture	3	0.081a[z]	1.43a
Undisturbed prairie	3	0.096a	1.23a

[z] Different letters after mean values represent significant differences (P< 0.05)
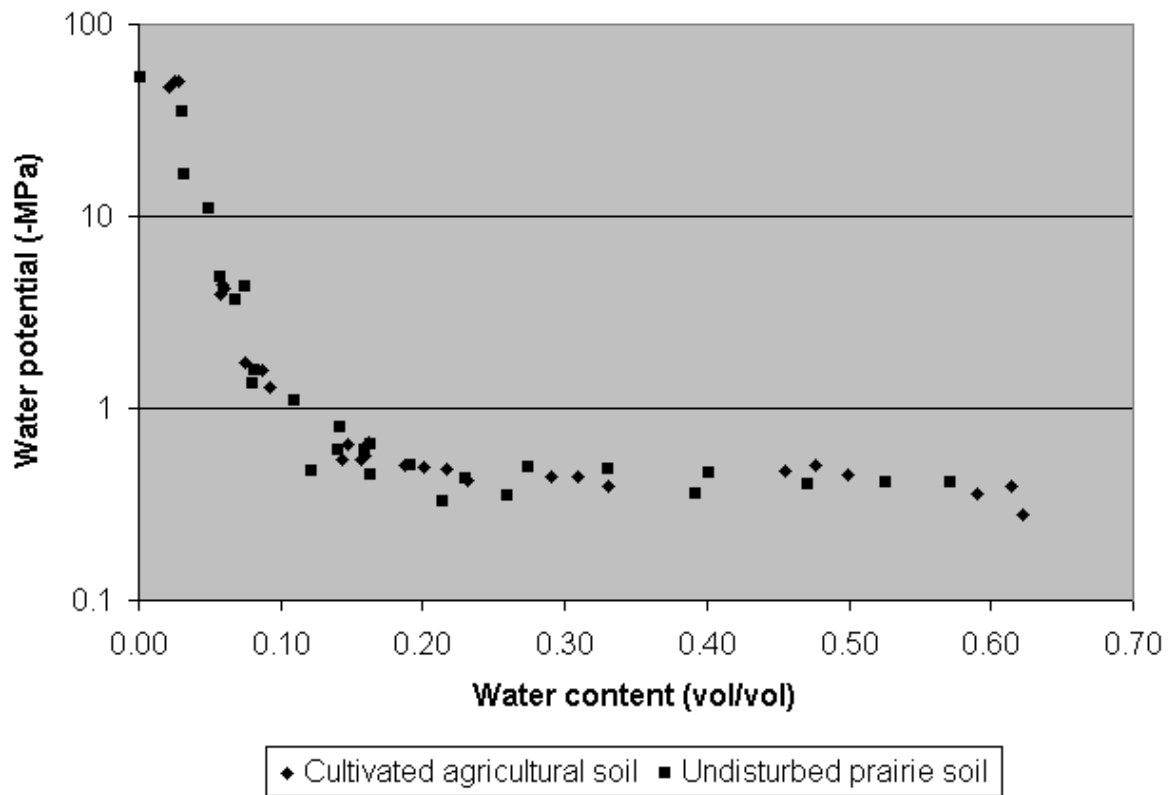
Fig. 2. The relationship between water potential, plotted on a log scale, and volumetric water content for a cultivated and undisturbed Jay silt loam soil in northwest Arkansas.

Expression patterns of novel wound-inducible plant genes in *Medicago truncatula*

Mandy M. Cox* and Kenneth L. Korth[§]

ABSTRACT

Terpenoids are an important class of defensive compounds that can accumulate in plants after pathogen infection or injury by chewing insects. Clones encoding putative terpene synthases and an oxidosqualene synthase, isolated from insect-damaged *Medicago truncatula* leaves, were selected from an expressed sequence tag (EST) database. The cDNA clones were used as radiolabeled probes to analyze gene expression in leaves treated by known factors that can trigger a defense response in plants. Transcript levels for all of the genes examined increased in response to artificial wounding, insect herbivory, and methyl jasmonate (meJA) treatments, whereas salicylic acid (SA) and glucose oxidase (GOX) had no measurable effects on transcript levels. Furthermore, the genome of *M. truncatula* was analyzed via DNA blots for an estimation of the number of copies of enzyme isoforms; these data indicate that each of the enzymes examined is encoded by a single-copy gene or a small gene family.

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[§] Kenneth L. Korth is an assistant professor in the Department of Plant Pathology.

MEET THE STUDENT-AUTHOR

Mandy Cox

I received a bachelor of science degree in food science in May 2002. The first half of my undergraduate study was done at Westark College, now known as the University of Arkansas at Fort Smith. My experience at Fayetteville has been challenging and rewarding. I am very grateful for the opportunities I have had to conduct research while still an undergraduate. During the summer of 2001, I accepted the C. Roy Adair Scholarship, which allowed me to initiate and complete this project. Despite being a food science student, I was drawn to the Department of Plant Pathology because of the molecular biology work being conducted there. My interests are in the areas of microbiology and molecular biology of food systems. This specific research project gave me hands-on training and additional knowledge that I could relate to plant foods. I will consider this experience beneficial as I continue my education for a master's degree in food microbiology at the University of Arkansas. I am still very excited about what I learned from this project and would like to thank all members of Dr. Ken Korth's lab for their help and friendship.

INTRODUCTION

Plants have unique responses to specific physical or chemical stimuli and this can be manifested by induction of genes putatively involved in a defense system. An example of a plant response to herbivory is demonstrated when lepidopteran larvae feed on a plant and volatile compounds are produced and systemically released from leaves. These volatile compounds can attract parasitoid wasps that are natural enemies of the herbivorous insect. In some cases wasps lay eggs in the lepidopteran larvae, and when they hatch, the wasp larvae devour the caterpillar from within (Turlings et al., 1995). Thus the plant is protected indirectly, through an induced mechanism, from further damage by the herbivore (Kessler and Baldwin, 2001).

One of the most abundant and common classes of induced volatile plant compounds released in response to insect herbivory is the terpenoids. For the induced production of volatile terpenoids, genes are possibly induced to express the proteins needed for volatile biosynthesis. The chemical pathways that lead to terpenoids begin with a five-carbon building block known as isopentyl diphosphate (IPP). Two independent biosynthetic pathways can produce IPP, the mevalonate (MVA) pathway localized in the cytosol and the 2C-

methyl erythritol 4-phosphate (MEP) pathway found in plastids. The five-carbon IPP units bond together in a head-to-head, head-to-tail, or head-to-middle fashion to form an acyclic prenyl phosphate. Specificity of the terpenoid produced is determined by the activity of the terpene synthase enzymes that convert the acyclic prenyl phosphate. Mono- and di-terpenes (C10 and C20 compounds, respectively) are thought to originate via the MEP pathway, whereas sesqui-, tri- (C15 and C30, respectively), and poly-terpenes are produced predominantly by the MVA pathway.

Terpenoids have a wide range of activities, and their applications range from flavorings and perfumes to pharmaceuticals. Individual sesquiterpenes have been shown to act as antimicrobial phytoalexins and as insect antifeedants. One form of triterpenes, known as saponins, have antifeedant and antifungal properties that aid in plant defense (Haralampidis et al., 2001).

A plant may respond differently to mechanical damage than to actual insect damage. These differential responses can be due to insect-derived oral factors that are perceived by the plant. A protein component in the saliva of some insects, glucose oxidase (GOX), may trigger a response by the plant (Felton and Eichenseer, 1999). Plant defense responses can also be triggered by the perception of volatile compounds such as methyl jas-

monate (meJA). Methyl jasmonate is produced constitutively in plants, but often increases in abundance as plants undergo stress. Plants release meJA, which serves as a signal to surrounding plants that an herbivore is feeding nearby. However, salicylic acid (SA), a key modulator of systemic acquired resistance (SAR) to pathogens, can interfere with the meJA-centered defense pathway (Felton et al., 1999).

For insect herbivory experiments, *Spodoptera exigua*, the beet armyworm, was employed to feed on plants. This caterpillar can be a serious pest of many crops such as alfalfa, bean, broccoli, corn, cotton, soybean, and tomato to list a few. Gene isolation and expression studies used *Medicago truncatula*, an excellent model plant for genetic analyses. This self-fertilizing legume possesses a small diploid genome and is easy to transform. It also has a relatively short generation time, allowing for more rapid genetic studies. In addition, a large scale genomics project in *M. truncatula* is underway, including the sequencing of expressed mRNAs. To date, over 140,000 expressed sequence tags (ESTs) are available in a public database (http://www.tigr.org/tdb/mtgi/); this includes nearly 10,000 ESTs that were derived from *S. exigua*-injured *M. truncatula*.

To understand plant defense responses to insects, it is important to characterize the regulation of genes that encode the enzymes which produce terpenes and saponins. Based on sequence similarities with known genes from other plant species, we selected three putative terpene synthase cDNA clones and one putative β-amyrin synthase (responsible for saponin biosynthesis) clone from the EST database. Accumulation of mRNA for each gene was measured in response to several types of wounding and treatment with meJA, SA, and GOX.

MATERIALS AND METHODS

Plant and insect maintenance

M. truncatula, line A17, was grown under standard conditions in a growth chamber at 24°C with a 16:8 hour light:dark regime. Fertilizer was administered at 2-week intervals. All treatments were carried out in a greenhouse and were started at 0900 hours. Eggs of *S. exigua* were obtained from the USDA Gast Rearing Lab (Starkville, Miss.). Larvae were maintained on an artificial diet under conditions at approximately 22°C.

DNA probes

DNA probes were derived from a cDNA library of *M. truncatula* leaves that had been subjected to *S. exigua* herbivory. The clones were identified in a search of the *M. truncatula* EST database based on sequence similarity with characterized terpene and beta-amyrin synthases

from other plant species. Four clones were chosen for analysis. The terpene synthase clones and their Genbank accession numbers, were: A4 (accession no. BF639687); A7 (accession no. BF640252); and A10 (accession no. BE321953). The putative β-amyrin synthase clone was designated B3 (accession no. BF642680).

Insect treatments

Insects were placed on plants and allowed to feed for 24 hours before samples were taken. Leaves that had been damaged by the insect were "local," and undamaged leaves on the same trifoliate as an insect-damaged leaf were "systemic." Artificial wounding was conducted by cutting leaves with scissors, and only the locally damaged leaves for this type of treatment were collected for sampling after 6 hours. Control samples came from leaves of undamaged plants.

Chemical treatments

For all meJA treatments, intact plants were placed in 18 L glass chambers. Cotton swabs with volumes of 0.5 μL, 1.0 μL, and 2.0 μL meJA (calculated volatile concentration 0.125μM, 0.25, and 0.5μM, respectively) were inserted into the soil next to each plant in separate chambers and the open-end bottom of each chamber was covered with a layer of foil and cheesecloth. A control plant was placed in a glass chamber with no added meJA. The plant was removed from the chamber after 1 hour and leaf samples were taken after 6 hours. A second experiment was conducted using the same technique but over an 18-hour time period.

Glucose oxidase was applied to leaves after wounding with a tracing wheel. As the wheel was rolled across the leaves, it punctured small holes in the plant, and 20 μL of 1.3 mg/mL GOX solution was pipetted onto the leaves. This level of GOX approximates the concentration measured in *Spodoptera* labial gland extracts (H. Eichenseer, personal communication). Local and systemic tissues were collected at 1 and 6 hours after the treatment. For testing the effects of SA, solutions of SA in water were sprayed on plants at 2mM, 4mM, and 8mM. For control treatments, the solvent with no SA was used. Samples were taken at 1 and 6 hours after treatment.

Nucleic acid analysis

Leaves were collected, immediately chilled in liquid nitrogen, and stored at -70°C until analysis. Total RNA was extracted using TriReagent (MRC, Inc. Cincinnati, Ohio) and separated on 1% agarose formaldehyde gels. The RNA was transferred to nylon membranes and hybridized with radiolabeled probes (Church and Gilbert, 1984). Insert DNA from individual cDNA clones was amplified via polymerase chain reaction and radiolabeled with 32P in random-primer reactions

(Sambrook et al., 1989).

M. truncatula genomic DNA was isolated according to Junghans and Mettzlaff (1990). DNA was digested in individual reactions with BamHI, EcoRI, or HindIII overnight at 37°C. Cleaved genomic DNA was separated on a 1.0% agarose gel, denatured, and transferred to a nylon membrane (Sambrook et al., 1989). Hybridizations were carried out as for RNA blots.

RESULTS AND DISCUSSION

EST clone selection

A search of the *M. truncatula* EST database revealed the presence of at least three putative terpene synthase clones and one β-amyrin synthase clone that were derived from insect-damaged leaves. Functional assignment of the clones was based on the presence of highly conserved sequence domains for each type of enzyme. Terpene synthase clones were designated A4, A7, and A10. The A4 sequence is predicted to encode a plastid transit signal at its amino terminus, indicating that it probably encodes a mono- or di-terpene synthase. The A7 and A10 clones bear highest sequence similarity to known sesquiterpene synthase clones, whereas the B3 clone is highly similar to characterized β-amyrin synthases. Consistent with their putative enzymatic functions, none of the A7, A10, or B3 sequences contains a predicted plastid transit sequence. Full-length cDNA clones for each sequence were obtained and utilized to characterize transcript accumulation and gene copy number.

Wounding induces transcript accumulation

Measurement of RNA accumulation demonstrates that genes encoding terpene synthases and β-amyrin synthase were induced by artificial- and insect-wounding. For most of the genes examined, the highest levels of transcripts are observed in leaves injured by insect herbivory (Fig. 1). For each gene, very low levels of RNA were present in undamaged leaves. Artificial damage also caused an increase in transcript accumulation, but generally not to the same degree as insect damage. This was not the case for A10, where the highest transcript levels were observed after artificial damage. For clones A7 and A10, two bands were consistently observed on RNA blots. This suggests there might be multiple forms of similar transcripts, derived from independent genes, that are cross-hybridizing on the membranes. For the A10 transcripts, the two bands were always observed at similar levels, suggesting that if they are derived from independent genes, these genes must be coordinately regulated. Transcripts for each gene were also induced in systemic tissues of insect-damaged plants, although not to the same level as in damaged leaves. The A7 transcript was consistently the most strongly induced of all the genes examined. Insect herbivory is known to often elicit a greater plant response than artificial damage, probably due to the differing types of wounding or the presence of elicitor compounds associated with the insect (Korth and Dixon, 1999; Walker-Simmons, et al., 1984). The enzyme products of the genes examined here are predicted to be involved in the biosynthesis of defense compounds, so it is not surprising that transcripts accumulated to high levels following wounding. Probing for the constitutively present ribosomal RNA indicated that equivalent amounts of total RNA were present in each gel lane.

Gene induction by methyl jasmonate

Treatment with meJA also led to transcript accumulation for each of the genes examined. Transcript levels were low in untreated samples, but RNA accumulation increased dramatically when plants are exposed to the lowest concentration of volatile meJA applied, 0.125μM (Fig. 2). Levels of A4 transcripts increased with increasing levels of meJA, whereas transcripts for the other genes were somewhat lower with increasing levels of meJA. Temporal expression of transcript accumulation was tested for the terpene synthase clones after exposure to volatile 0.25 μM meJA (Fig. 3). The A4 transcripts were present to some degree even in untreated control samples in this experiment, but the RNA blot seemed to indicate that transcripts accumulated to higher levels between 2-6 hours after initial exposure to meJA. For A7 and A10, transcript levels clearly increased with time, and returned to normal levels by 18 hours after the initial exposure. As in wound experiments, the A7 transcripts were the most abundant, and the A7 transcripts also were induced earlier than the other genes tested.

Glucose oxidase and salicylic acid treatments

Glucose oxidase is the most abundant protein found in labial gland saliva of lepidopteran insect larvae (Eichenseer and Felton, 1999). This enzyme has been shown to affect plant responses to chewing caterpillars and to wounding when it is applied to a wound site (J. Bede and G. Felton, personal communications). Addition of GOX did not have any effect on transcript accumulation for any of the genes used in this study; levels of RNA in leaves treated with GOX did not differ significantly from those treated with water (data not shown).

In addition, treatment with SA ranging from 2-8 mM did not affect transcript accumulation (data not shown), therefore SA alone seems not to be directly involved in the regulation of these genes.

Enzymes encoded by small gene families

Probing *M. truncatula* genomic DNA with the selected cDNAs revealed that this species contains low copy numbers of the genes examined. Banding patterns indicate that the terpene synthase sequences are present in one to three copies each, whereas sequences hybridizing to the B3 β-amyrin synthase clone are present in three to four copies (Fig. 4). Although the genes examined here all encode well-conserved protein domains, it is well established that enzymes similar in primary sequence can differ greatly in terms of the specific products that they synthesize (Bohlmann et al., 1998). Therefore, independent genes that cross-hybridize on DNA blots might encode enzymes with very different specificities. At the very least, our data indicate that there is a low degree of genetic redundancy for the sequences that we tested via genomic DNA blots. Knowledge of the copy number of these genes will be important if efforts are made to isolate genomic promoter sequences.

The results from our experiments reveal that insect herbivory and chemical treatments can cause systemic gene responses. Systemic leaves of wounded plants accumulated transcripts for terpene and β-amyrin synthases, showing that gene-induction signals are being transported through the plant. In wounded leaves, the highest levels of transcript accumulation were generally observed after insect herbivory. This result is indicative of the presence of specific insect-derived elicitors or a unique type of damage during chewing by lepidopteran larvae as compared to mechanical wounding.

Treatments with meJA, a central modulator of wound responses in most plant species, showed that this plant hormone can regulate expression of defense genes in *M. truncatula*. Addition of volatile meJA to intact plants caused a rapid and transient accumulation of terpene synthase- and β-amyrin synthase-encoding genes.

The addition of GOX and SA, compounds known to affect expression of some plant defense genes, did not affect accumulation of any of the genes examined in this study. Although GOX can repress levels of some plant-defense gene transcripts (J. Bede and K. Korth, unpublished data), we did not see any differences in transcript levels when comparing wounded leaves with and without added GOX.

Understanding the regulation of the genes described here might aid ultimately in manipulation of plant defense responses or in the biosynthesis of valuable terpenoid compounds. The role of these genes' products in defense is suggested by the strong and rapid induction of transcripts that occurred following insect herbivory. With the basic characterization reported here, targeted studies of the function of these genes in defense can be carried out. The enzymes that these genes encode, or the promoter sequences that control their regulation, might provide valuable tools in production of plants that are more insect-resistant. This work clearly demonstrates that *M. truncatula* can serve as a new source of novel and valuable genes encoding enzymes involved in plant defense and terpenoid biosynthesis.

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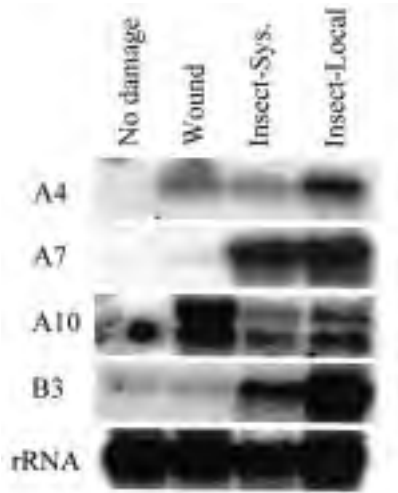
Fig. 1. Transcript accumulation as indicated by RNA blots, in leaves following artificial damage with scissors ("wound"), or *S. exigua* herbivory. Leaves were collected at 6 hours after the initial damage. Membranes were hybridized with the indicated probes, and bands were visualized via autoradiography.
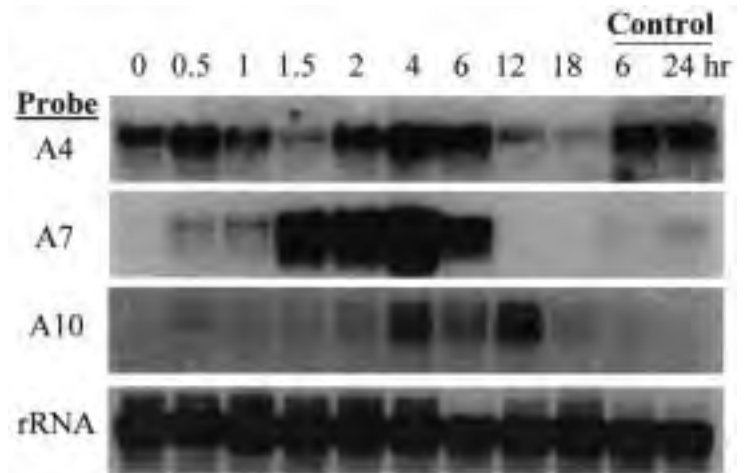

Fig. 3. Transcript accumulation in leaves following exposure to 1.0 µl (0.25 µM) of meJA. Leaves were collected at the time indicated, after intact plants were placed in a glass chamber for 1 hour with meJA. Membranes were hybridized with the indicated probes, and bands were visualized via autoradiography.

Fig. 2. Transcript accumulation in leaves following exposure to differing levels of meJA. Leaves were collected 6 hours after intact plants were placed in a glass chamber with 0, 0.5 µl (0.125 µM), 1.0 µl (0.25 µM), or 2.0 µl (0.5 µM) meJA for 1 hour. Membranes were hybridized with the indicated probes, and bands were visualized via autoradiography.
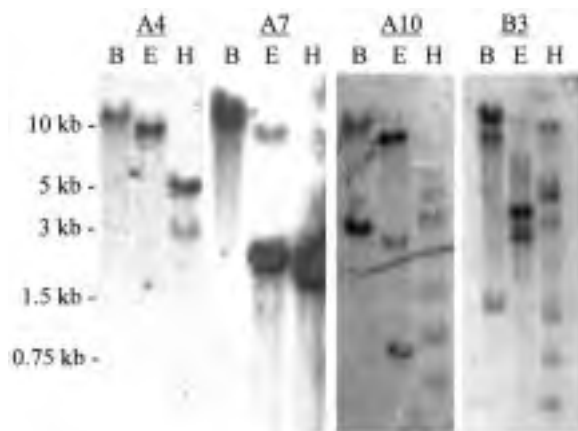

Fig. 4. DNA blot analysis of terpene synthase and β-amyrin synthase clones. Genomic DNA from *M. truncatula* was digested with *Bam*HI (B), *Eco*RI (E), or *Hind*III (H) and separated on a 1% agarose gel. Positions of DNA size markers are indicated at left. Identical membranes were hybridized with the indicated probes, and bands were visualized via autoradiography.

Quantification of land-use impact on stream water quality

Scott Dennis*, Indrajeet Chaubey§, Brian E. Haggard†

ABSTRACT

Accelerated eutrophication of Beaver Lake in northwest Arkansas is a major environmental concern. When developing watershed-management plans to protect lake water quality, it is important that linkages among land-use activities and water quality of tributary streams be quantified. This study assessed longitudinal base-flow and storm-flow water quality at War Eagle Creek and quantified linkages between stream water quality and land-use conditions within the War Eagle Creek sub-watershed of the Beaver Lake watershed. We collected six water samples: three from base-flow conditions and three from storm-flow conditions during Spring 2002. In general, concentrations of nitrate nitrogen (NO_3-N), total N (TN), total organic carbon (TOC), conductivity, and total dissolved solids (TDS) increased as the sampling moved downstream. All stream water-quality parameters, except phosphate phosphorus (PO4-P), were significantly correlated to the ratio of agricultural-to-forest land-use ($r^2 = 0.90$ to 0.97). These results indicate that the ratio of agricultural-to-forest land-use within the watershed can be used to evaluate stream water quality, and that increases in this ratio may result in increased TDS, NO_3-N, TN, and TOC concentrations.

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I graduated in May 2002 with a major in biological engineering. I also attended high school in northwest Arkansas where baseball was a major part of my life. I planned to play baseball in college. I visited many colleges that wanted me to play for their team, however, none had a major in which I was interested. An acquaintance of mine told me about biological and agricultural engineering and immediately I knew that discipline is what I would rather focus on.

In my career as a student at the University of Arkansas, I have worked with teams in many design projects. I participated in an experiment where we built a growth chamber to test the affects of global warming. I was on a team that designed a sensor that would detect water clarity and participated in the design of an infrared switch for a feed bin in a chicken house that shuts an auger off when a bin is full in order to eradicate feed spills. I also helped develop and build a chicken controlled retractable chicken cage that allowed a chicken to determine the optimal area needed in order to reduce psychological stress.

I specialized my degree in environmental aspects, thus allowing me to research a river that not only is important to the welfare of northwest Arkansas but also is important to me. My family owns a farm through which the river flows. I enjoy nature, and I feel it is important to protect natural areas throughout the nation.

I am a member of the American Society of Agricultural Engineers (ASAE). ASAE allows me to continue my education in the newest developments and latest technologies in the agricultural industry.

Scott Dennis

INTRODUCTION

Nonpoint source (NPS) transport of nutrients, sediment, and pathogens from agriculturally dominated watersheds is a major concern in Arkansas (Edwards and Daniel, 1992; Edwards et al., 1997). There is ample evidence to suggest that row crop agriculture and excess land application of animal manure have led to surface and ground-water pollution (Edwards et al., 1996). Increasingly, watersheds are unable to utilize and degrade the high levels of inorganic fertilizers and animal manures applied to the landscape. The result is increases in noxious oxygen-consuming and sometime toxic algal blooms, deteriorations of fisheries, and general degradation of water quality (Park et al., 1994; Sharpley et al., 1994). The Arkansas 303(d) list (list of waterbodies not supporting their designated use) for 2002 includes 59 stream segments totaling 1,269 miles and five lakes totaling 17,062 acres of impairment. Nine of the stream segments, totaling 70 miles, are located on small streams dominated and impacted by point-source discharges (ADEQ, 2002).

In northwest Arkansas, concern over non-point-source (NPS) pollutants entering War Eagle Creek from the application of animal waste and inorganic fertilizers has been increasing. War Eagle Creek is a major tributary to Beaver Lake, which provides drinking water to Northwest Arkansas. The water quality of War Eagle Creek directly affects Beaver Lake, and accelerated eutrophication from nitrogen (N) and phosphorus (P) in Beaver Lake is a major concern. Thus, it is imperative that the water quality of tributary streams be assessed so that watershed management plans can be developed. The agricultural land-use in the War Eagle Creek watershed is dominated by poultry growers and beef operations; therefore, animal manure is the main source of fertilizer for these farmers. Even though animal manure is a good source of nutrients, excess land application may result in runoff losses of nutrients and lead to accelerated eutrophication of downstream waterbodies.

To develop a watershed management plan for the Beaver Lake watershed and to protect the lake water quality, the linkages among land-use activities and stream water quality must be understood. Similarly, there is a need to survey current land-use practices in the watershed so that areas contributing the majority of nutrients can be identified. Currently, data are not available to quantify the changes in base-flow and storm-flow water quality in the War Eagle Creek sub-watershed in relation to land-use conditions; however Haggard et al. (2002) observed increasing nutrient concentrations and export with increasing pasture land-use throughout the Beaver Lake watershed.

Quantification of linkages between land-use and water quality is needed to determine the sources of pollution in streams. Accurate identification of the NPS pollutants involved is also required for designing best management practices (BMPs) for stream and lake water-quality protection.

The objectives of this study were to:

a. Assess longitudinal base-flow and storm-flow water quality of War Eagle Creek at five water-quality sampling stations.
b. Quantify linkages between stream water quality and land-use in the War Eagle Creek sub-watershed.

MATERIALS AND METHODS

This study was conducted in the War Eagle Creek sub-watershed, located within the Beaver Lake watershed (Fig. 1). The total area of the War Eagle Creek sub-watershed is approximately 264 mi². The principal land-uses in the War Eagle Creek sub-watershed are forestry and agriculture, which cover 61% and 38% of the total watershed area, respectively. Urban land-use within the War Eagle Creek sub-watershed covers less than 1% of the area.

To quantify the effect of land-use on water quality, five water-quality sampling stations were established at War Eagle Creek. The locations of the sampling stations were based on site accessibility while keeping them as equidistant as possible. Station 1 was the most upstream station at War Eagle Creek, and Station 5 was the most downstream (Fig. 1).

Three water samples were collected during base-flow conditions at two-week intervals between February 2002 and April 2002 at each of the five sampling sites. In addition, water samples were also collected during three storm events. At each site, three filtered samples were collected by filtering 20 mL of stream water using 0.45 μm nylon membrane filter in the field. Three unfiltered water samples, each 500 mL in volume, were also collect-
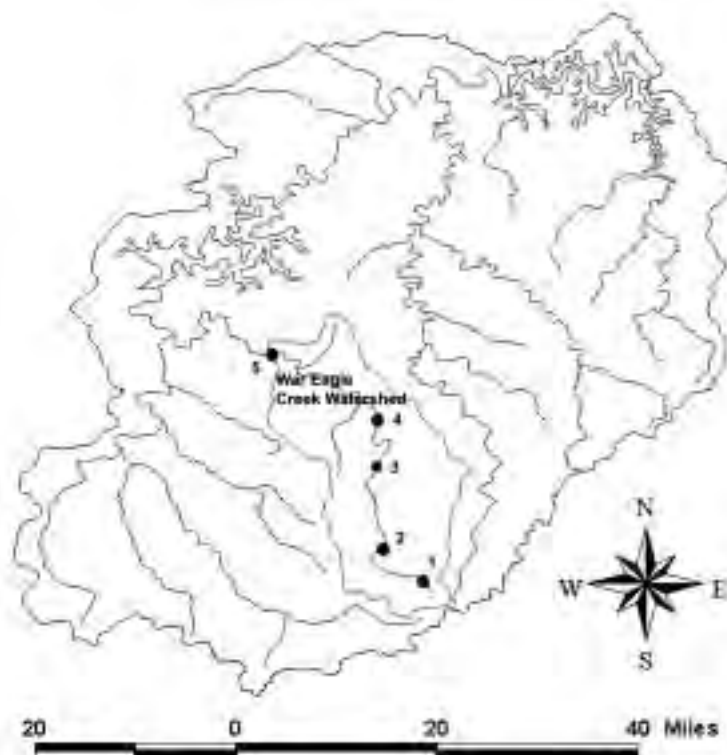

Fig. 1. Location of the War Eagle Creek sub-watershed and water-quality sampling stations within the Beaver Lake basin.

ed at each sampling station. Bottles and filtering syringes were field-washed prior to sample collection. Immediately after collection, water samples were stored on ice in the dark until returned to the laboratory. Samples were transported to the laboratory for analyses of dissolved P (PO_4-P), nitrate N (NO_3-N), total N (TN), and total organic carbon (TOC). We also measured pH at each site using a pH meter (Oktron Corporation, model pH Testr 2), and conductivity, salinity, and temperature using an YSI conductivity, salinity, and temperature meter (YSI Corporation, model YSI85).

We measured dissolved P with an autoanalyzer using ascorbic-acid reduction and NO_3-N using cadmium-copper reduction methods. Total N and TOC were measured by converting all N and organic C into nitrogen oxide (NO) and carbon dioxide (CO2) at 950°C, respectively. These gases were analyzed by infrared detection of CO2 and conversion of NO to nitrogen dioxide (NO2) and followed by NO2 decay measurement by a photo multiplier probe.

The topographic or digital elevation model (DEM) and land-use data for the War Eagle Creek sub-watershed were obtained from the Center for Advanced Spatial Technology (CAST), University of Arkansas, and analyzed using ArcView GIS. The sub-watersheds draining toward the each sampling station were delineated using DEM data and the ArcView GIS. Sub-watershed boundary for each sampling station was then intersected with the War Eagle Creek sub-watershed land-use data to calculate fraction of each land-use type within each sampling station sub-watershed (Table 1).

Table 1. Land-use and area of each sampling station sub-watershed within the War Eagle Creek sub-watershed.

Land-use	Watershed areas under different land use (mi^2)				
	Station 5	Station 4	Station 3	Station 2	Station 1
Residential	1.60	0.28	0.02	0.00	0.00
Agriculture	100.64	39.65	20.40	4.97	0.91
Forest	161.16	115.89	86.68	31.06	4.44
Other	0.22	0.16	0.14	0.05	0.00
Total area	263.62	155.98	107.24	36.09	5.35

The land-use data were used to estimate the agricultural to forest ratio within each sub-watershed. The linkages among stream water quality and land-use were determined by regressing the measured water-quality data for each sub-watershed against the ratio of agriculture to forest area within the sub-watershed.

RESULTS AND DISCUSSION

Table 2 shows the average water quality data, for three dates at each sampling station for the base and storm-flow conditions. In general, concentrations of NO_3-N, TN, TOC, conductivity, and TDS increased as sampling

moved downstream in the sub-watershed, during both base-flow and storm-flow conditions. The only exception to this trend was PO_4-P concentration, which decreased at sampling Stations 3 and 4 during base-flow and was highly variable during storm-flow conditions. The variability in PO_4-P during storm-flow conditions may be attributed to transport of particulate matter, which may adsorb PO_4-P during the transport process.

Stream pH did not change significantly among sampling stations. Temperature data are difficult to compare for this study because they are a function of the time of the day and degree of shading by riparian vegetation. Since temperature at all the sampling stations was not collected during the same time of the day, it is difficult to infer trends from upstream to downstream locations.

When we compared the water-quality data during base-flow and storm-flow conditions at each sampling station, the data indicated that concentrations of NO_3-N, TN, and TOC were higher during storm-flow conditions (Table 2). The PO_4-P concentrations were higher during storm-flow conditions at sampling Stations 1, 2, and 3, and lower at Stations 4 and 5. Concentration of TDS was higher during base-flow conditions at Stations 1 and 2. Conductivity values were lower during storm-flow conditions at all the sampling stations except Station 4. This may have been due to dilution of stream conductivity/TDS concentrations during storm-flow conditions.

Total flow of nutrients is a function of concentration and flow volume. Flow volume is always larger during storm-flow events. A higher concentration during storm-flow conditions indicates that a significantly larger amount of nutrients may be transported downstream in War Eagle Creek, eventually entering Beaver Lake during rainfall events.

Table 2. Average data collected from War Eagle Creek. There were 3 storm-flow and 3 base-flow sampling events.

Parameter	Station 1	Station 2	Station 3	Station 4	Station 5
Storm-flow condition					
pH 8.2	8.2	8.2	8.4	8.2	
Temp (°C)	12.1	11.4	9.1	8.1	8.6
Conductivity(mS/cm)	30.3	43.0	70.6	100.2	130.5
TDS (mg/L)	13.7	19.7	32.0	47.3	64.3
NO_3-N (mg/L)	0.23	0.35	0.64	1.02	1.52
TN (mg/L)	0.31	0.41	0.66	1.08	1.33
PO_4-P (mg/L)	0.020	0.036	0.009	0.009	0.033
TOC (mg/L)	1.04	1.13	0.95	1.35	1.76
Base-flow condition					
pH 8.5	8.2	8.2	8.3	8.2	
Temp (°C)	12.8	12.4	11.4	11.0	11.8
Conductivity(mS/cm)	45.9	53.1	63.9	94.7	159
TDS (mg/L)	19.0	23.3	28.3	35.0	59.0
NO_3-N (mg/L)	0.21	0.25	0.34	0.44	1.06
TN (mg/L)	0.26	0.25	0.36	0.39	0.89
PO_4-P (mg/L)	0.007	0.029	0.007	0.024	0.048
TOC (mg/L)	0.58	0.58	0.88	1.10	1.39

To quantify the linkages between land-use and stream water quality, a linear regression was performed between agriculture-to-forest-area ratio and stream water-quality concentration for each sampling station. Agriculture-to-forest-area ratio gives an indication of the dominance of agricultural land-use in the watershed with regard to the degree of watershed development. The ratio ranged from 0.16 (Station 2) to 0.62 (Station 5). In general, as the sub-watershed area increased, the dominance of agricultural land-use also increased. Concentration of stream water-quality parameters as a function of agriculture-to-forest-area ratio for each sampling station is shown in Fig. 2. An increase in the agriculture-to-forest-area ratio resulted in increased concentrations of NO_3-N, TN, TDS, and TOC.

The linear regression showed that all stream water quality parameters, except PO_4-P, were significantly related to agriculture-to-forest-area ratio within the sub-watershed (Table 3). The coefficient of determination (r^2) values ranged from 0.90 – 0.97 and were highly significant ($p < 0.01$). Only 33% of the variability in the stream PO_4-P concentration could be accounted for by the ratio of agriculture-to-forest-area, which was not significant ($p = 0.31$). This was contrary to the expected role of agricultural land-use in controlling stream PO_4-P concentrations; however, annual mean PO_4-P concentra-tions have shown an increasing relationship with pasture land-use across the entire Beaver Lake watershed (Haggard et al., 2002). More data need to be collected in this watershed to further verify this relationship.

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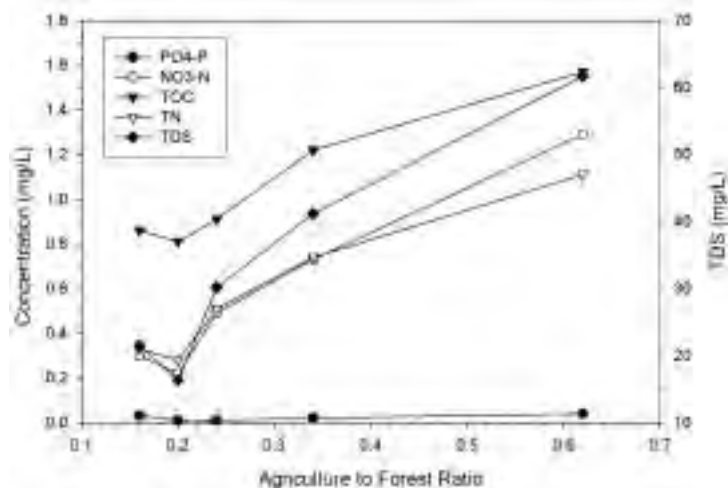
Fig. 2. Concentration of stream water-quality parameters as a function of agriculture-to-forest-area ratio in the sub-watershed.

Table 3. Linear regression results to predict War Eagle Creek water quality as a function of agriculture-to-forest-area ratio.

Water quality parameter	Regression equation[1]	R^2	P
NO_3-N (mg/L)	Y = 2.292 X - 0.109	0.97	< 0.01
TN (mg/L)	Y = 1.801 X + 0.032	0.95	< 0.01
TDS (mg/L)	Y = 94.36 X + 4.72	0.90	< 0.01
TOC (mg/L)	Y = 1.696 X + 0.546	0.95	< 0.01
PO_4-P (mg/L)	Y = 0.044 X + 0.009	0.33	0.31

1 Y = stream water quality (e.g. NO_3-N concentration); X = agriculture-to-forest-area ratio

Organophosphate toxicosis in chickens: A case report

Robert Hubbard*, Judith England[§], and F. Dustan Clark[†]

ABSTRACT

Two cases of organophosphate toxicity were diagnosed at the University of Arkansas Poultry Science Department poultry research farm in the Spring of 2002. In both cases the birds were being treated with the organophosphate RaVap® for Northern Fowl Mites (*Ornithonyssus sylvarium*) infestations. A total of 61 birds died and 13 were treated successfully with atropine sulphate.

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[§] Judith England is the Project/Program Manager for the Poultry Science Department poultry research farm.

[†] F. Dustan Clark, faculty sponsor, is Extension Poultry Health Veterinarian in the Poultry Science Department.

INTRODUCTION

Organophosphate compounds are commonly used as contact insecticides and acaracides for many animals and plants. The major classes of organophosphates are phosphates, phosphorothionates, phosphorothiolothionates, phosphorothiolates, phosphoramidates, pyrophosphates, phosphonates, and phosphoramides (Hatch 1978).

Since these chemicals are widely used, they may be a source of animal poisoning in a variety of ways. Feeds may be contaminated at the processing plant or during shipping, storage, or handling on the farm. Feed crops may be dusted with insecticide, or the harvested crop may become accidentally contaminated. Animals may be sprayed or dusted with too much insecticide and may become overdosed. Also, water sources may be contaminated by spillage or by rinsing out spray tanks and hoses. Occasionally, the source of contamination cannot be determined (Hatch, 1978).

In most animals, acute toxicosis from organophosphates is due to an irreversible inhibition of acetylcholinesterase (ACh-E) wherever acetylcholine (ACh) functions as a transmitter (Reigart and Roberts, 1999. Fraser and Mays, 1986). The result of ACh-E inhibition is accumulation of ACh in neuromuscular junctions; parasympathetic postganglionic terminals in smooth muscle, cardiac muscle, and glands; in all autonomic ganglia; and in cholinergic synapses within the central nervous system. This causes over-stimulation of the cholinergic receptors by ACh. If the overstimulation is intense enough, the ACh receptors may become blocked. However, complete blockage of all cholinergic receptors is usually not produced, and early clinical signs reflect hyperfunction of these receptors. Lethal amounts of organophosphates cause death from a number of effects of nicotinic, muscarinic, and central cholinergic receptor over-stimulation and/or paralysis. The animal eventually dies of asphyxia (Hatch, 1978).

If the exposure is not too severe, then the animal may not die. In fact animals have been known to spontaneously recover from organophosphate poisoning. ACh-E is usually not 100% blocked since the enzyme is constantly being produced by neurons. Even though the animal can recover, it may still take several days or weeks for an animal to recover completely from a sublethal dosage (Hatch, 1978).

Female chickens with organophosphate toxicity show significant alterations in the brain, spinal cord and peripheral nerves. Axons in the brain stem, ventral and lateral tracts of the spinal cord, in gray matter of the spinal cord, and in the sciatic nerve become swollen and

MEET THE STUDENT-AUTHOR

I graduated from Harrison High School in 1999 and began college at the University of Arkansas the following fall semester as an undecided major. The next summer I became aware of the Poultry Science Department and decided to look into becoming a poultry science major. I quickly decided to begin pursuing a degree in poultry science. Since then I have been introduced to different types of research in the poultry field and have had opportunities to do some research of my own. I am now a senior and plan to graduate in May of 2003 with a B.S.—in poultry science with a minor in music. I am an active member in the Razorbacks for Christ and the Poultry Science Club.

I chose this project based on my fascination with diseases and microorganisms. I thank Dr. Wayne Kuenzel for introducing me to research and Dr. F. Dustan Clark for his guidance throughout this project.

Robert Hubbard

fragmented with the heaviest damage occurring in the spinal cord. Cell bodies in these areas also sustain significant damage. Organophosphate toxicity also causes an effect known as organophosphorus-induced delayed neurotoxicity (OPIDN), which is characterized as a "dying back" of axons. The degeneration of the myelin and axonal portions of the long axons in the spinal cord, peripheral nerves, and medulla usually accompany OPIDN. Syndromes causing nerve damage other than OPIDN have also been known to occur (Carrington et al., 1988).

During a study on the effects of massive oral dosages of Rabon®, a popular organophosphate insecticide, six groups of chicken hens were given dosages of Rabon® daily. Hens given more than 188 mg/kg of Rabon® became inactive and lethargic by the third day of treatment. Birds given higher amounts of 752 mg/kg and 1504 mg/kg had a decrease in weight and food intake, and developed loose and sometimes bloody droppings. Birds given 1504 mg/kg were unable to stand, had whitish dry combs, and sat trembling with their heads down and eyes closed. All birds given 1504 mg/kg died by the seventh day of treatment and sixty percent of the birds given 752 mg/kg died by the fourteenth day (Yadava et al, 1970).

In an experiment using Cyanophenphos (0-ethyl-0-cyanophenyl phenyl phosphonothionate), which causes delayed neurotoxicity similar to that of neurotoxic organophosphorus compounds, chicken hens with high doses (80-540 mg/kg) became paralyzed, deteriorated rapidly in body weight, and eventually died of ataxia. It was observed that hens receiving higher doses became fatigued and unwilling to walk. Clumsiness and unsteadiness of gait followed with muscle tone continuing to diminish and flaccidity progressing within 12-24 days. Eventually complete flaccid paralysis developed and the birds showed no sign of recovery (El-Sabae et al, 1980).

MATERIALS AND METHODS

Our article includes two case reports describing instances of organophosphate toxicity in chickens associated with the treatment of Northern Fowl Mites (*Ornithonyssus sylvarium*) with the product RaVap®. Clinical symptoms, lesions, associated mortality, and therapy are described.

CASE REPORTS

Case 1. In March 2002 approximately 500 birds housed at the University of Arkansas Center of Excellence for Poultry Science research farm, Fayetteville, were treated with the organophosphate.

RaVap® (tetrachlorvinphos 23%, dichlorvos 5.7%. Boehringer Ingelheim Vetmedica, Inc. St. Joseph, MO.) for Northern fowl mites. The birds were of various genetic crosses and purebred standard poultry used to study poultry genetics. The birds were housed in floor pens (approximately 1.53 meters by 3.84 meters) with a total of 28 pens in the house. The birds were fed a standard poultry ration with the younger birds on a developer ration. All birds were watered from a standard bell type (Plasson waterer) with one waterer per pen. The birds had been diagnosed earlier with Northern fowl mites. a poultry parasite which can cause anemia, feather loss, and skin damage. The treatment for the mites consisted of RaVap® solution applied to each bird in the house. The poultry farm uses a rotating chemical external parasite control program consisting of RaVap®, Sevin® 80% WP, and a 10% permethrin compound. The schedule was such that RaVap® was the compound to be used at this time. The RaVap® solution was prepared according to label instructions by mixing 1/2 gallon of the product in 25 gallons of water. This solution is a 0.6% solution. The birds were caught and submerged (up to the head) in the RaVap® solution. The house was kept warm (approximately 27-30 degrees Centigrade). Standard ventilation was used in the house during dipping of the birds. The birds were treated on a Friday morning. Clinical symptoms appeared within 4-6 hours in a few birds being described as listless. By Monday morning a total of 56 birds were dead and another 7 birds had varying degrees of clinical symptoms and lesions. Ataxia, depression, anorexia, and closed eyes were the most common symptoms; two of the seven birds were almost comatose. It was noted that of the 56 birds that died over the weekend; 27 died Friday night. Treatment consisted of daily injections of 1/120 grain of atropine sulphate (Amtech, Phoenix Scientific Inc St. Joseph, MO) subcutaneously. A dosage of 0.5-1.0 ml was used and body weight was estimated. The dosage used corresponds to a dosage of 1 ml per 7.5 pounds of body weight. Injections were given for 3 days and birds were examined for a total of 5 days. Affected birds recovered in 3 days with the two most severely affected birds recovering in 5 days. The most severe mortality was seen in the purebred Australorp (three males) and Australorp-Smyth line crosses (27 birds). The three Australorp males lost represented 50% of the Australorp males treated for mites.

Case 2. In May 2002 approximately 288 female chickens and 96 male chickens housed at the Center of Excellence for Poultry Science research farm, Fayetteville, were treated with the organophosphate Ravap®. for Northern Fowl Mites (Ornithonyssus sylvarium) infestation. These chickens were from the Ascites

and random-bred genetic lines maintained for genetic studies. The female chickens were housed in standard-size Leghorn wire cages and the males were housed in broiler breeder male wire cages. Nipple-type drinkers were used in the male cages and cup-type drinkers were used in the female cages. The birds were fed standard breeder ration. The RaVap® solution that had been used was prepared according to label instructions by mixing 1/2 gallon of the product in 25 gallons of water producing a 0.6% solution. The birds were treated for mites by spraying them around the vent area until that area was thoroughly wet. Overnight four of the birds sprayed died and another seven developed clinical symptoms. The symptoms were similar to those seen in the other chickens in that the affected birds had varying degrees of depression, closed eyes, ataxia, and appetite loss. However, some of the affected chickens had a reddish discoloration of the shanks of the legs. The treatment of these surviving birds consisted of daily subcutaneous injections of 1/120 grain of atropine sulphate. The dosage was 1.0-1.5 ml and again body weight of the chickens was estimated. The dosage used corresponds to a dosage of 1 ml per 7.5 pounds of body weight. Injections were given for three days and birds were examined for a total of five days. One of the seven affected birds died but the remaining six recovered. The five total birds that died were all males. No females developed clinical symptoms of organophosphate toxicity.

RESULTS AND DISCUSSION

The exact reason for the toxic effects in the birds in these two case reports could not be determined. The Ravap® was mixed in accordance with the label instructions according to personnel involved in treating the chickens with the insecticide. If the product was mixed incorrectly it can be speculated that more birds would have been affected.

The Australorp males and Australorp-Smyth line crosses suffered the highest mortality of all Ravap® treated birds in the first case report. Three of the six purebred Australorp males died from the Ravap® treatment (representing a 50% loss of purebred males treated). This suggests that the Australorp breed of chicken may have a greater sensitivity to the Ravap®. This latent sensitivity to organophosphates may be related to genetics, stress, or other variables. In the practice of veterinary medicine, it is reported that some pets have a skin reaction to flea collars (Fraser and Mays, 1986) and occasionally certain breeds of dogs have other reactions to some types of flea collars. This reaction often is manifest by the dog appearing tired, sluggish, and having periods

of vomiting; the dog may recover uneventfully when the collar is removed (Dustan Clark, personal communication).

In the second case report, five male birds treated with Ravap® died and two other males showed symptoms of organophosphate toxicity and then recovered. These five birds were random-bred broiler-breeder males and were the first birds of the males sprayed for mites. It had been speculated earlier that these birds may have received a higher level of Ravap® since they were sprayed first and as such the product may not have been thoroughly mixed. However, it was determined that the Ravap® was mixed correctly and, in fact, the hens in the second case report were sprayed before the males. Therefore, these first five males would in actuality have been birds 289 through 293 sprayed from the spray tank since 288 hens were sprayed prior. Since only males died in the second case report, it is possible that there may be a sex-specific sensitivity to the ingredients in Ravap®.

A total of 61 chickens died in both case reports following either dipping or spraying with Ravap®. In the first case report, 27 of the chickens died the first night and 29 more died over the remainder of the weekend. In the second case report four birds died overnight. A total of 14 chickens in both case reports were treated with atropine sulphate for clinical symptoms consistent with organophosphate toxicity. Atropine sulphate was an effective treatment since only one of the 14 birds treated failed to respond to the atropine sulphate injections. Fig.1 depicts a bird with clinical symptoms of Ravap® toxicity; fig. 2 depicts a chicken that has responded to atropine sulphate therapy. It is possible that more of the chickens could have been saved (especially in case report one) if the adverse reactions had been reported to the Poultry Science Department veterinarian immediately so atropine therapy could have been started sooner.

The exact reasons for the animal's toxicity from the Ravap® may never be determined from these two cases. Since organophosphate products can be toxic it is important to mix the products in accordance with label instructions and use them appropriately. It is also important to report any adverse symptoms in animals after product use to a veterinarian immediately so treatment can be administered.

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Fig. 1. A male chicken exhibiting clinical symptoms of organophosphate (RaVap®) toxicity.

Fig. 2. A recovered male chicken (top left) after atropine sulfate therapy in a pen with another male and two females.

The influence of storing high-moisture content rough rice on milling quality

Julita M. Manski* and Terry J. Siebenmorgen§

ABSTRACT

The objective of this research was to determine the influence on drying characteristics of storing high-moisture content (MC) rough rice under various conditions and durations before drying. Two cultivars of rice, 'Bengal', a medium-grain cultivar, and 'Cypress', a long-grain cultivar, were used. The MC of 'Bengal' was 24.8%[1] and that of 'Cypress' was 20.4% at harvest. Immediately after harvest, drying runs were performed with samples of both cultivars under two drying air conditions: one at 51.7°C (125°F) and 25% relative humidity (RH), and the other at 60°C (140°F) and 17% RH. Storage treatments using the high MC rice were also initiated immediately after harvest. Both cultivars of rough rice were stored for one month (i.e., 27 d) and three months (i.e., 76 d) in either a walk-in freezer at –9°C (15°F), a household refrigerator at 3.5°C (38.3°F) or a walk-in cooler at 4°C (38.5°F). After one month and three months of storage, all samples were dried under the same two drying air conditions as at harvest. The head rice yield (HRY) was determined for all the dried samples. There were no differences in the HRYs of samples that were stored for one or three months and then dried and in those HRYs of samples dried immediately after harvest; this finding was consistent across the three storage temperatures for both cultivars. The trends in HRY reduction were similar to previously reported drying trials using these drying air conditions. This research indicates that it is possible to store rough rice at high MCs for up to three months under storage temperatures varying from –9°C to 4°C without affecting HRY.

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§ Terry J. Siebenmorgen, faculty sponsor, is a professor in the Department of Food Science.

[1] All moisture contents are expressed on a wet basis unless otherwise noted.

MEET THE STUDENT-AUTHOR

Although I was born in Poland, I have lived in the Netherlands since I was four. After graduating from high school in 1997, I wanted to pursue a major in which science as well as engineering subjects were integrated. Food science and technology seemed the perfect major comprising my fields of interest. The only university in the Netherlands offering this major with a master's degree was in the small college town of Wageningen. Therefore, my decision to attend Wageningen University was easily made.

In addition to attending classes and labs during the first three years of the education, I was also involved in our Food Science Club. I have worked several part-time jobs throughout my college time as well. During the last two years, I was able to put my gained knowledge into practice by completing two theses in the field of process engineering.

Since internships are required in my major, during the fall of 2001 I had the opportunity to conduct an internship at the University of Arkansas. This internship was one of the best experiences of my college time. Both the research that I executed, and the experience of living in the USA were very instructive. I would like to take the opportunity to thank Dr. Terry Siebenmorgen for advising me during the research, and Jerry Fendley for helping me carry out the experiments. Further, I want to express my gratitude to every one that I met, for making my stay in Arkansas precious. Having been abroad has given me the chance to develop myself both professionally and personally.

In the summer of 2002 I will conduct a second internship. Arrangements are being made by H.J. Heinz Company in Pittsburgh, Penn., to work with the R&D department. I will graduate from Wageningen University in November 2002. After graduating, I hope to enroll in a Ph.D. program at a university in the USA.

Julita Manski

INTRODUCTION

Immediately after harvest, on-farm and commercial rice driers as well as research laboratories conducting rice drying research face a busy drying season. All would benefit tremendously if the possibility existed to delay drying by storing rough rice at high MC for a period of time prior to drying. High-MC storage of rough rice under specified conditions could be a means to extend the drying season, provided the properties and thus the drying characteristics of the rice remain unchanged during storage.

Previous research has mainly focused on storing rough rice under different conditions varying from 2°C to 38°C after drying (Chrastil, 1990; Daniels et al., 1998; Kitamura et al., 1977; Pearce et al., 2001; Villareal et al., 1976). These studies showed that storage temperature had a significant effect on various rice quality indices. Daniels et al. (1998) showed that rice storage MC also played an influential role. The storage of rough rice at high MCs has received little attention.

MATERIALS AND METHODS

Harvest

Two cultivars of rice, 'Bengal' and 'Cypress', were harvested at the Rice Research and Extension Center, Stuttgart, Ark., in August 2001. Immediately after harvest, the rice was transported to the University of Arkansas Rice Processing Laboratory, Fayetteville, Ark., and cleaned using a Carter-Day Dockage tester (Carter-Day Co., Minneapolis, MN). Upon arrival at the lab, the MC of 'Bengal' was 24.8% and the MC of 'Cypress' was 20.4%. Bulk sample MCs were determined by drying 15 to 16 g of rough rice in a convection oven for 24 h at 130°C (Jindal and Siebenmorgen, 1987). Individual kernel MC measurements were performed using an individual kernel moisture meter (Model CTR-800 E, Shizuoka

Seiki Co., Ltd., Shizouka, Japan). After cleaning, samples of both cultivars were immediately dried and others were dried after certain storage durations.

Storage

Immediately after harvest, lots of 'Bengal' and 'Cypress' rough rice were placed in sealed plastic containers in one of three different storage environments represented by storage temperatures of –9°C (15°F), 3.5°C (38.3°F), and 4°C (38.5°F). The -9°C temperature was attained by placing containers in a walk-in freezer, that of 3.5°C was maintained in a household refrigerator, and that of 4°C was maintained in a walk-in cooler. It is to be noted that the original experimental design specified a higher temperature to be maintained in the refrigerator. However, controls were such that the average temperature was 3.5°C; thus the treatments in the walk-in cooler and refrigerator are included as essentially replications. From an economic point of view, the above-freezing conditions might be realizable for the industry to temporarily store rough rice. The -9°C storage condition, however, was included as a possible storage environment for research samples.

Each of the six sub-lots of rough rice (two cultivars x three storage environments) were stored in sealed plastic containers, each containing approximately 23 kg. The rice was stored for two durations, one month (27 d) and three months (76 d), in each of the three storage environments. After one month and three months of storage, approximately 8 kg of each of the six stored lots were taken out of the containers and equilibrated for 5 to 12 h at room temperature before drying.

The actual temperatures of the storage environments were monitored during the study and are shown in Fig. 1. The temperatures for the storage environments for the walk-in freezer (-9°C) and the walk-in cooler (4°C) were consistent during the storage duration. However, the temperature in the refrigerator (3.5°C) fluctuated due to the inability of the refrigerator to adjust to changes in ambient temperature; the average of temperature readings during the three month storage was 3.5°C.

Drying

Immediately after harvest, samples of both cultivars were dried using two air conditions. Both drying air conditions were chosen based on previous research (Fan et al., 2000). The first condition was 51.7°C and 25% relative humidity (RH) and is representative of actual conditions used in commercial drying. The resulting equilibrium moisture content (EMC) as predicted by the Chung equation was 7.3% (ASAE, 1998). The second condition was 60°C and 17% RH. This condition is at the upper extreme of commercial drier temperature levels, but was shown to have potential for use if combined with a tempering treatment (Cnossen and Siebenmorgen, 2000). The resulting EMC for the second drying air condition was 5.8%. These two conditions were used for all drying runs. Each drying run for a cultivar / drying air condition / storage duration / storage temperature was performed twice.

The first drying runs conducted immediately after harvest represent the drying characteristics of rice that was not stored prior to drying. These runs will be referred to as the drying runs of month 0. After one
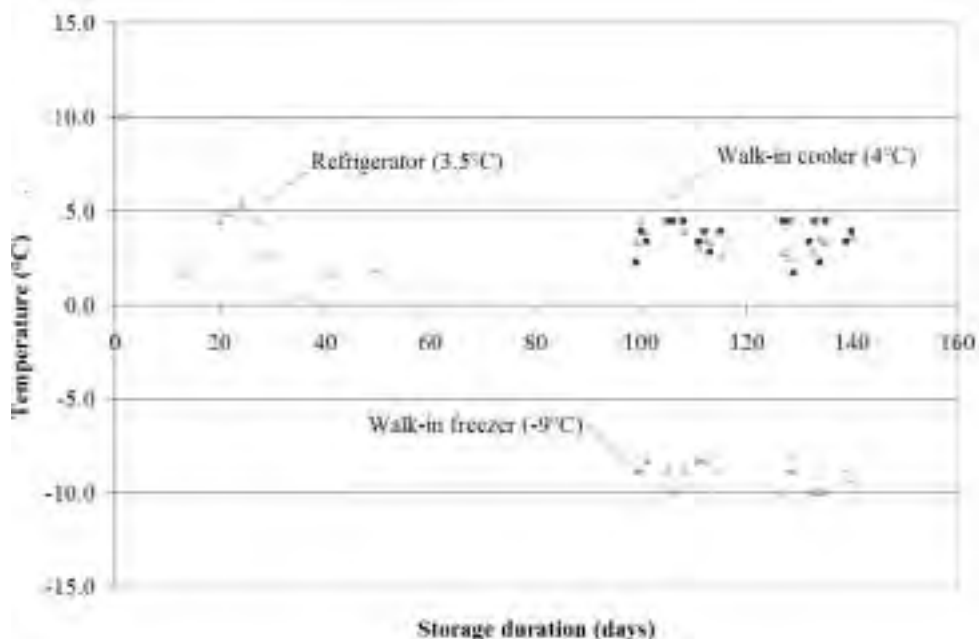

Fig. 1. Actual temperature histories of the three storage environments.

month and three months of storage, samples of the stored rough rice were equilibrated to a lab temperature of approximately 25°C and then dried under the same drying air conditions as month 0.

The drying air conditions were controlled by temperature and RH control units (Parameter Generation & Control, Inc.,-PG & C-Black Mountain, NC) and monitored by a Hygro-MZ dew-point monitor (General Eastern, Woburn, MA). Air from each PG&C unit was supplied to laboratory drying chambers, each consisting of 16 trays (15 ¥ 25 cm) with perforated bottoms. The 16 trays were arranged as two eight-tray sets. In each of the eight-tray sets, one tray was designated to be weighed at each defined drying duration to measure the weight loss due to drying. Each tray was filled with a uniform layer of approximately 110 g of rice. The drying durations for a drying run were 0, 10, 20, 30, 45, 60, 90, and 120 min. Drying durations were randomly assigned to trays to eliminate the influence of the location of a sample in the drying chamber. After a certain drying duration, paired trays from each eight-tray set were combined to form one rice sample for milling. After drying, the combined samples were immediately placed in a conditioning chamber (21°C, 48% RH) to cool and to slowly continue to dry to an MC of approximately 12.5%. This cooling is known to produce a reduction in HRY if a sufficient MC gradient is present in the kernel (Cnossen and Siebenmorgen, 2000). After conditioning for four to five days, the samples were stored in sealed plastic bags in a cooler at 4°C for one to two months prior to milling.

Milling

Upon removal from cold storage, samples were first equilibrated to room temperature before hulling and milling. Approximately 150 g of dried rough rice was hulled with a Satake Rice Machine (Satake Engineering Co., Ltd., Tokyo, Japan). The resultant brown rice was milled with a laboratory miller (McGill No. 2, Rapsco, Brookshire, TX). A weight of 1.5 kg was placed on the lever arm of the mill 15 cm from the centerline of the mill chamber. All samples were milled for 30 s. The milled samples were aspirated with a South Dakota Seed Blower (Seedburo, Chicago, IL) for 30-60 s to clean the rice by removing any bran that was left after milling. The weight of head rice was determined with a Graincheck 2312 Analyzer (Foss Tecator, Höganäs, Sweden). Head rice comprises kernels that are at least three-fourths of the original kernel length. HRY was then calculated as the weight percentage of rough rice that remained as head rice after milling.

For a selection of samples, the degree of milling (DOM) of the head rice was determined with a milling meter (Satake MM 1B, Satake Engineering Co., Ltd., Tokyo, Japan). Since the rice samples were not physically separated into head rice and broken kernels by the Graincheck, the head rice from the samples was separated with a double-tray shaker table (Grainman, Grain Machinery Mfg., Miami, FL) before determining the DOM. The range of DOM for 'Bengal' head rice was 73 to 90 with an average of 82. 'Cypress' head rice showed a higher DOM range of 99 to 115 with an average of 102.

RESULTS AND DISCUSSION

Overall Observation

Physical differences were observed between the rough rice samples that had been stored before drying and those that were dried immediately after harvest. The rough rice held at –9°C showed ice crystals between the rice kernels after three months of storage. However, no ice crystals were observed after one month of storage. Since 'Bengal' was stored at a high MC of 24.8%, visible mold growth was expected and confirmed after one and three months of storage at 3.5°C and at 4°C. At the storage temperature of –9°C no mold growth was found on 'Bengal' or 'Cypress' at any storage duration.

The MC of the stored rice samples was measured immediately after each storage duration. The MC of both cultivars increased only slightly during storage: the maximum increase in MC for 'Bengal' was 0.5 percentage points and for 'Cypress' 0.9 percentage points. Immediately after harvest and after one and three months of storage, individual kernel MC measurements were performed. The expectation was that the MC distribution would become narrower after storage. However, there was no observable difference between the kernel MC distribution of the freshly harvested rice and the stored rice. These results were consistent for both cultivars stored at all temperatures.

Head Rice Yield (HRY)

HRY trends of 'Bengal' and 'Cypress' samples that were dried immediately after harvest (Fig. 2) closely resembled those of previous research (Fan et al., 2000). When drying under the air condition of 51.7°C and 25% RH, the HRY for 'Cypress' remained almost constant, even for extended drying durations. The HRY of 'Bengal' showed a decrease for this same air condition as the drying duration exceeded 20 min before cooling to 21°C. Since 'Bengal' is a medium-grain rice cultivar, its features include a short and thick kernel. Previous research has shown that this type of kernel is more susceptible to fissuring after drying than long-grain cultivars such as 'Cypress' that comprise long, thin kernels (Fan et al.
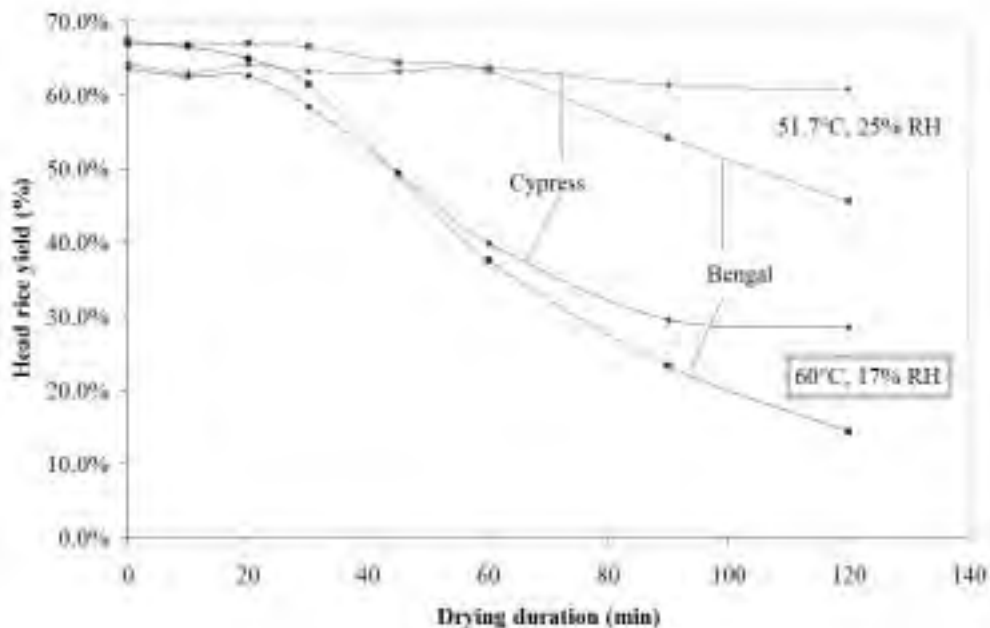
Fig. 2. Head rice yields for 'Bengal' and 'Cypress' versus drying duration, for the case in which drying was performed immediately after harvest (month 0) under the two indicated drying air conditions.

2000; Jindal and Siebenmorgen, 1994; Kunze, 1983). The more severe drying air condition of 60°C and 17% RH caused a greater decrease in HRY for both 'Bengal' and 'Cypress' than did the lower-temperature drying condition. The greater decrease of 'Bengal' HRY over that of 'Cypress' was more apparent after 20 min of drying for both drying air conditions. Further, when drying 'Cypress' under the severe drying air condition of 60°C and 17% RH, the HRY approached a constant value after 90 min of drying regardless of further increase in drying duration. The results in Fig. 2 are the reference values to compare HRYs of samples that had been stored prior to drying.

The HRY trends of the rice samples that had been stored for one and three months at the three storage environments of –9°C, 3.5°C, and 4°C before drying were similar to the HRY response of the month 0 samples. This was observed for both cultivars (Figs. 3 and 4). The storage durations did not influence the HRY response of the samples, especially in the first 30 min of drying under both drying air conditions. Since drying durations of 20 to 40 min are common in the industry, these results are very promising in regard to possible commercial application.

In order to compare the HRY responses of the three storage environments, the average HRYs over the three storage durations were computed for each storage environment (Fig. 5). It is very clear that there were no differences in HRY trends observable between the three storage environments. Even freezing of rough rice for up

to three months and then drying did not affect the HRY compared to rice that had been dried immediately after harvest. The physical degradations that were observed during storage, such as ice crystals in the frozen samples and growth of mold in the higher temperature storage environments, did not apparently influence the HRY of the rice when dried.

This research shows that high-MC rough rice can be stored for up to three months at temperatures varying from below to just above freezing without affecting HRY when dried. Although physical changes such as mold growth did occur during storage of the high MC rough rice, there was no apparent effect on milling quality due to the mold growth. For commercial driers this finding indicates that the drying season could possibly be extended, which would provide more flexibility. On a laboratory scale, the findings indicate that drying trials do not have to be conducted immediately after the harvest of rice but can be performed after storing temporarily in cold storage. It is emphasized that further research focusing on the effects of such storage practices on other quality factors is required before industrial implementation.
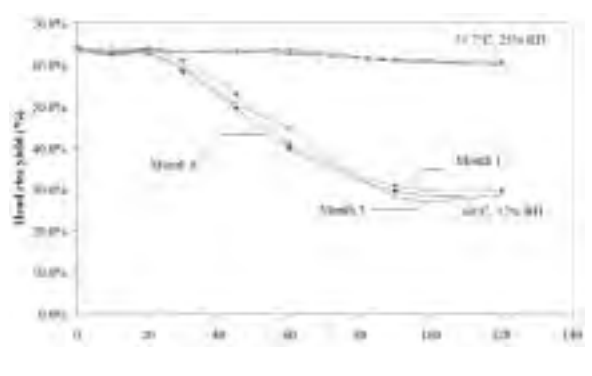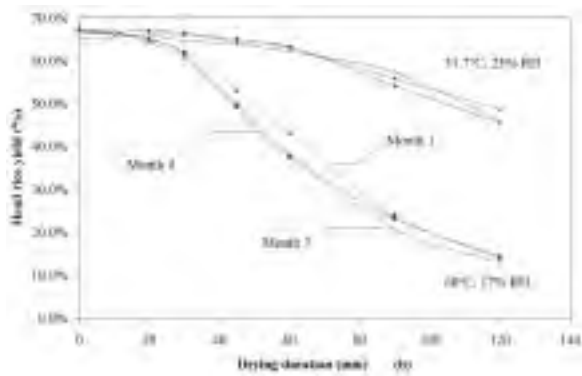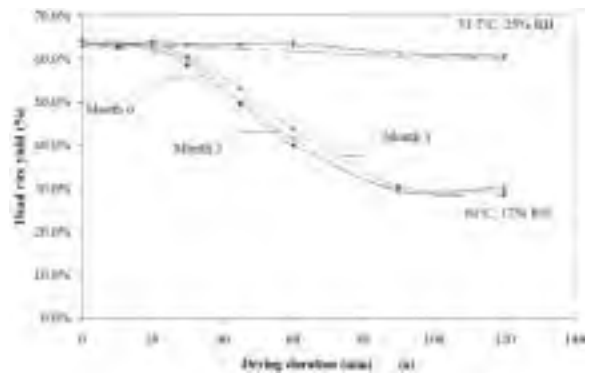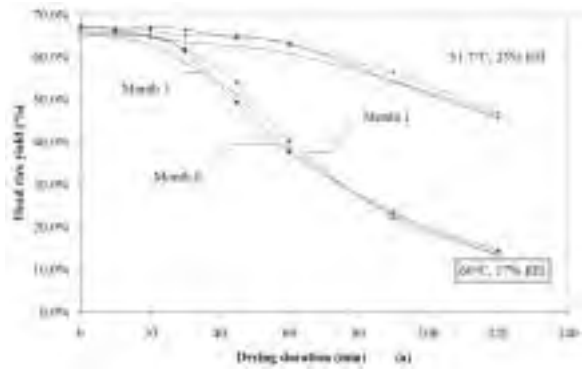
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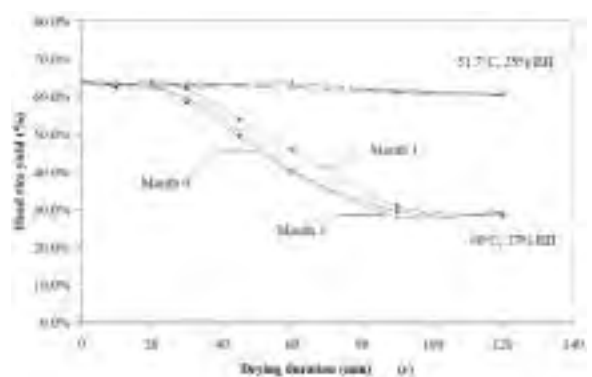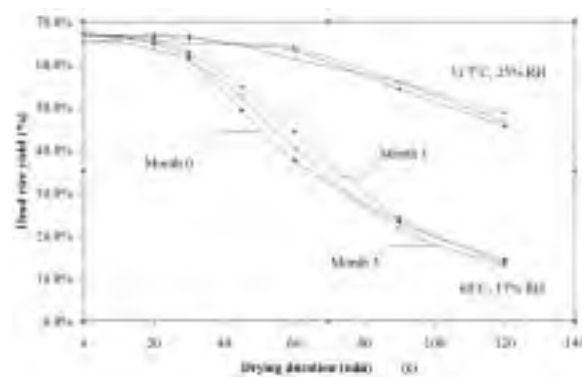
Fig. 3. Head-rice yield response of 'Bengal' stored in a freezer at –9°C (a), in a cooler at 4°C (b), and in a refrigerator at 3.5°C (c) for the indicated durations before drying with the two indicated drying air conditions versus the drying duration.

Fig. 4. Head rice yield response of 'Cypress' stored in a freezer at –9°C (a), in a cooler at 4°C (b), and in a refrigerator at 3.5°C (c) for the indicated durations before drying with the two indicated drying air conditions versus the drying duration.
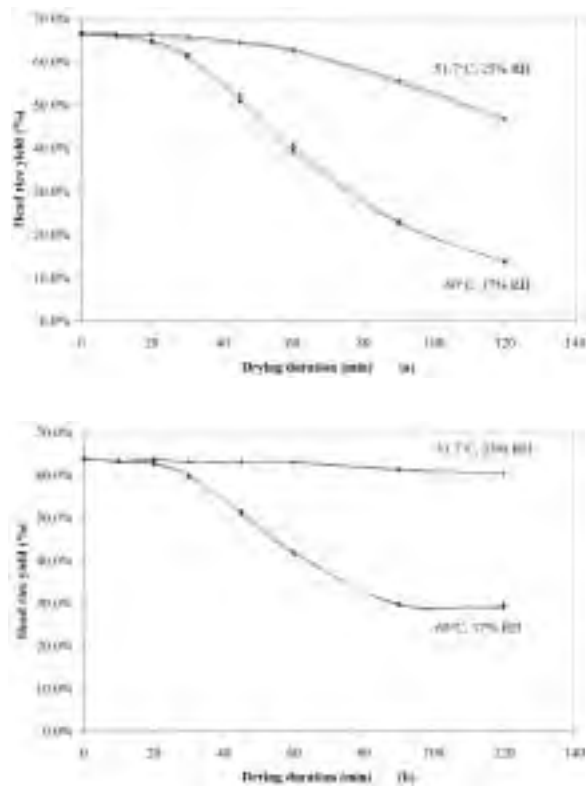
Fig. 5. Average head rice yield response over the storage durations of 'Bengal' (a) and 'Cypress' (b). The various curves represent the three storage environments of a walk-in freezer (-9°C), a refrigerator (3.5°C), and a walk-in cooler (4°C).

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Bradyrhizobium japonicum and soybean symbiotic response to glyphosate in glyphosate-tolerant soybean

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ABSTRACT

Soybean (*Glycine max*) grain contains approximately 40% protein and 6.5% nitrogen (N) on an elemental basis. Therefore, the plant requires an abundant N supply throughout its life cycle, and symbiotic N fixation of soybean with *Bradyrhizobium japonicum* provides 40 to 85% of the soybean N. Although soybean cultivars have been genetically engineered to withstand the herbicide glyphosate, *B. japonicum* grown in culture is sensitive to glyphosate. We hypothesized that glyphosate applied to glyphosate-tolerant soybean would inhibit nodulation by *B. japonicum* unless *B. japonicum* could also be selected for glyphosate tolerance. Cultures of *B. japonicum* were challenged with sublethal doses of glyphosate, and individual colonies were selected for growth in the presence of glyphosate. Of the 40 isolates that were originally selected for glyphosate tolerance, all isolates in subsequent experiments had similar sensitivity to glyphosate as wild-type *B. japonicum*. To determine if glyphosate affected *B. japonicum* in plants, soybean seeds were imbibed with differing levels of glyphosate and water and then planted and inoculated with *B. japonicum*. After several weeks of growth the plants were harvested and nodules were scanned and analyzed by digital imagery. Glyphosate application to glyphosate-tolerant soybean did not affect the ability of *B. japonicum* to form nodules and fix nitrogen. These data do not agree with previous responses of small soybean plants sprayed with glyphosate, which showed delayed nodulation and decreased nodule size. It may be that the dosage applied to plants and the timing of the application affect the response of glyphosate on symbiotic effectiveness.

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INTRODUCTION

In the legume family, plants form a symbiotic relationship with bacteria in the soil. In this relationship the bacteria infect the roots of the plant and chemically reduce (fix) nitrogen (N_2) gas from the atmosphere into a form that the plant can use. In return, the plant provides the bacteria with a source of carbon and an appropriate environment for bacterial growth. The morphological structure that results from the bacterial infection of the plant root is called a nodule, and this is where N fixation occurs. Nitrogen fixation does not occur until about three weeks after the bacteria infect the plant root when large, irregular shaped nodules with a red interior indicate that N fixation is occurring (Graham, 1998).

Soybean is a member of the legume family and forms this symbiotic relationship with *Bradyrhizobium japonicum* (Harper, 1987). Nitrogen fixation by this bacterium is very important to the production of soybean and

allows farmers to produce soybean in the absence of costly N fertilizer.

Because soybean seed contains a large amount of protein, the plant must be supplied with an abundant N supply throughout its life cycle, and bacterial N fixation provides 40 to 85% of the soybean N requirement (Graham, 1998). Past research has shown that *B. japonicum* strains differ in their ability to form nodules and in how well they fix N. Selection for superior N-fixing *B. japonicum* has been accomplished, and in controlled environments, these strains demonstrated increased N fixation (Vasilas and Fuhrman, 1993). However, in field environments these bacteria have not been effective in increasing N fixation and yield. This is due to existing *B. japonicum* populations in the soil. These indigenous strains out-compete the superior strains and form over 90% of the nodules (Johnson et al., 1965). The indigenous *B. japonicum* strains are often inefficient at N fixation compared to the superior strains, and full yield potential may not be realized.

Recent advances in biotechnology have led to the engineering of soybean cultivars that are tolerant to the chemical glyphosate. Glyphosate is the active ingredient in the non-selective herbicide Roundup™ (Duke, 1988). These glyphosate-tolerant (GT) cultivars allow for better post-emergence weed control in soybean fields. Glyphosate works by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme in the plant that leads to the synthesis of aromatic amino acids. Glyphosate-tolerant soybean expresses a gene for EPSPS that is tolerant to the herbicide. Extensive research under field conditions has shown that yields of glyphosate-tolerant soybean sprayed with glyphosate are comparable to glyphosate-sensitive cultivars in conventional herbicide systems (Delannay et al., 1995).

Although engineered soybean cultivars are tolerant to glyphosate, the N-fixing bacteria that form the symbiotic relationship with the soybean are not tolerant to glyphosate (Jaworski, 1972). In culture, *B. japonicum* growth is inhibited by glyphosate, depending upon concentration of glyphosate in the culture and the sensitivity of the bacterial strain. Glyphosate-tolerant soybean plants do not readily degrade glyphosate, and it concentrates in metabolic sinks such as roots and nodules (Duke, 1988). Since the bacteria live in these areas, the concentration can have a negative effect on their growth, and research has shown that early application of glyphosate to plants delays N fixation and nodulation (King et al., 2001). The observation that glyphosate delays nodulation by *B. japonicum* may provide a clue as to how to increase the competitiveness of superior N-fixing *B. japonicum* over poor N-fixing indigenous strains.

We hypothesized that glyphosate applied to GT soybean would inhibit nodulation by *B. japonicum* unless *B. japonicum* could also be selected for glyphosate tolerance. A practical corollary of this hypothesis is that *B. japonicum* selected for glyphosate tolerance would be more competitive for nodulating GT soybean seedlings treated with glyphosate, which could increase the competitive advantage of superior N-fixing *B. japonicum* strains over inferior indigenous strains. In this experiment our objectives were to determine the effect of glyphosate applied as a seed-imbibition solution, to evaluate the effect on nodulation with wild type *B. japonicum*, and to select for GT *B. japonicum* by challenging existing strains with glyphosate.

MATERIALS AND METHODS

Greenhouse Experiment One

Glyphosate solutions of 50, 25, 12.5, 6.25, and 3.125 mM were prepared using serial dilution of a 50 mM

solution. Six petri dishes were filled with a single layer of GT soybean cultivar DK5961RR seed, and seeds were weighed. Each petri dish then received 20 mL of one of the glyphosate treatments or water. Several hours after imbibition, 15 mL of each solution was added to ensure there was free solution in the bottom of each petri dish. After the overnight soaking, the seeds were blotted with paper towels and weighed.

B. japonicum (strain USDA 110) was cultured in a defined medium that lacked amino acids and included arabinose as the carbon source (Karr and Emerich, 1989). During mid log-phase, a culture was diluted with deionized water to an optical density at 600 nm (OD600) of 0.0654 (approximately 3.08×106 cells/mL, Mahler and Wollum, 1981), which served as inoculum.

Pots (15 cm diameter) were filled with N-free potting medium (LB2, Sungro Horticulture, Bellevue, Wash.) and inoculated with 1 mL of *B. japonicum* culture followed by 500 mL of –N nutrient solution (deSilva et al., 1996). Six seeds were planted in each pot, and there were six replications arranged in a randomized complete block design. Greenhouse lights were set for a 15-hour photoperiod from 6 am to 9 pm and provided a minimum of 300 umol PAR cm-2s-1 at plant height. Greenhouse temperatures were approximately 28 Å 3%C (day) and 22 Å 2%C (night). After 1 week, very poor germination was observed in all pots, indicating that this effect was not due to glyphosate. Cotyledons appeared small, yellow, and damaged, and plants were discarded. Due to the observance of poor germination in all treatments, a germination study was performed to find the best method of imbibition.

Germination/Imbibition Study

Two methods of seed imbibition were compared to planting dry seed. The first method involved placing a piece of filter paper in the bottom of a petri dish and then placing the seed on top of the filter paper. The filter paper was kept moist for the 3-hour imbibition. For the second method, seeds were placed in a petri dish, and the petri dish was kept half filled with water for the 3-hour period. Forty seeds were used per treatment, and both wet and dry weights were taken. After imbibition, four replications of 10 seeds from each imbibition treatment were planted and compared to a control treatment of planting dry seed. These data clearly indicated that imbibition on moist filter paper was superior to imbibition in a partially filled petri dish (Table 1). These results are discussed in more detail in the Results and Discussion section.

Greenhouse Experiment Two

After deciding to use the filter paper imbibition method, Greenhouse Experiment one was repeated.

Plants were harvested after 4 weeks of growth in the greenhouse. Shoots were harvested above the soil line and placed in a 65%C dryer. Roots were also harvested, and the nodules on the roots were separated into two groups, <2.36 mm and >2.36 mm, with a mesh sieve. Each group of nodules was scanned and then placed in the 65%C dryer. Dry weights were then taken of roots, shoots, and nodules. Nodule scans were made with a flatbed scanner and nodule number determined from the images using Sigmascan Pro (V. 5.0, SPSS Inc., Chicago, Ill.).

B. japonicum Selection for Roundup Resistance

A culture of USDA 110 was grown in defined media with arabinose as its carbon source and NH4+ as its N source (Karr and Emerich, 1989). Therefore, synthesis of proteins would require de novo amino acid production, including a functional EPSPS, which is the target enzyme inhibited by glyphosate. One hundred μL of the culture was plated out on defined media containing 10 mM glyphosate. One hundred individual colonies were selected from the agar plate and were grown in 5 mL of liquid culture (minus glyphosate). Liquid cultures were adjusted to an OD600 of approximately 0.16, and 100 μL was added to 5 mL of liquid media containing 5 mM glyphosate. Wild-type USDA 110 in the presence and absence of glyphosate was used as a control. The cultures were allowed to grow for 14 days, and then the OD600 of each culture was measured.

Greenhouse Experiment Three

The results from Greenhouse Experiment Two were used in designing Greenhouse Experiment Three with several modifications. In Greenhouse Experiment Three, seven different glyphosate-tolerant cultivars were used. The cultivars were: USG 540NRR, Progeny 5415RR, Delta Grow 5450RR, Asgrow AG5603, Asgrow AG5901, Delta King 5661RR, and Delta King 5961RR. Three different glyphosate treatments were used: 12.25, 3.06, and 0 mM glyphosate. Plants were harvested 23 days after planting.

RESULTS AND DISCUSSION

Germination/Imbibition Study

Germination was affected by imbibition treatments

Table 1. Response of seedling emergence and seedling damage to imbibition treatments.

Imbibition treatment	Emergence %	Damaged seedlings %
3 hours partially submerged	35	35
3 hours filter paper	80	38
Dry seed	98	15
LSD[z]	20	N.S.

[z] LSD = least significant difference (P≤0.05); N.S. = nonsignificant.

(Table 1). Germination was significantly decreased by imbibing seeds in a petri plate that was partially filled with water. Germination of seeds that were imbibed on wet filter paper was not significantly different from that of dry seeds. The amount of damaged seedlings did not differ significantly between any of the treatment groups. Due to these results, the filter-paper method was chosen for use in Greenhouse Experiment Two.

Greenhouse Experiment Two

Glyphosate concentration did not affect nodulation or plant dry weight (Table 2). There were no significant

Table 2. Plant dry weight, nodule number, and nodule size response to glyphosate seed treatments in Greenhouse Experiment Two. There were no significant effects (P≤0.05) for any treatments for any variables.

Glyphosate concentration --(mM)--	Nodule number Large	Small	Nodule weight Large	Small	Dry weight Roots	Shoots
			--------(mg)-------		---------(g)----------	
0	23	23	15.6	80.2	0.32	1.32
3.13	26	23	15.5	97.3	0.33	1.33
6.25	31	31	10.6	90.1	0.40	1.42
12.5	29	25	13.3	84.6	0.36	1.26
25.00	25	24	16.1	74.0	0.39	1.23
50.00	29	32	13.0	77.6	0.35	1.15

differences among any of the treatments for average nodule weight, nodule number, or dry weights of other plant components.

B. japonicum Selection for Glyphosate Resistance

Glyphosate inhibited growth of all bacterial strains

Table 3. Growth of *B. japonicum* isolates in the culture containing 5 mM glyphosate.

Strain	Glyphosate	OD600
USDA 110	-	1.33 a[z]
USDA 110	+	0.44 b
Selected[y]	+	0.36 b

[z] Means followed by the same letter within a column are not significantly different (P = 0.05).
[y] Forty individual cultures of USDA 110 were selected based upon their ability to form colonies on agar media containing glyphosate. There were no significant differences among selected strains, and an OD600 is presented that was averaged over all strains.

(Table 3). The cultures that were selected from the agar media containing glyphosate did not show growth that was significantly different from the wild type USDA 110 grown in the presence of glyphosate. The selected cultures also did not show differences among strains selected for glyphosate tolerance. The growth of the selected cultures in the presence of glyphosate was significantly less than that of the wild type USDA 110 grown in the absence of glyphosate. USDA 110 grown in the presence of glyphosate also showed growth that was significantly less than that from USDA 110 grown in the absence of glyphosate.

Greenhouse Experiment Three

There was no significant interaction of cultivar and glyphosate treatment in the study; alsp, there were no significant differences among any of any of the cultivars in response to glyphosate treatments for plant dry weight, average nodule weight, or average nodule number. There were significant differences among cultivars,

Glyphosate content in the seed and young plant after 3 hours imbibition would expectantly range from approximately 60 to 500 µg as the concentration of glyphosate in the imbibition solution increased from 6 to 50 mM. In comparison, two sequential foliar applications of glyphosate at 1.12 kg ha-1 at the unifoliolate and first trifoliolate stages (King et al., 2001) would deliver approximately 730 µg per plant, half of which would be absorbed by the plant. An additional possibility for lack of response of glyphosate in an imbibing solution on nodulation is that the glyphosate would be primarily absorbed into the cotyledons, which may not transport glyphosate as readily to developing roots as would glyphosate delivered to leaves.

The rationale for imbibing seed in a glyphosate solution was that it would affect the infection and nodulation process from the initial stages of germination, beginning with radicle emergence from the seed. It was hypothesized that this treatment would affect nodula-

Table 4. Plant dry weight, nodule number, and nodule size response to glyphosate seed treatments in Greenhouse Experiment Three. Values reported are averaged over glyphosate treatments (cultivar x glyphosate, interaction non-significant, $P \leq 0.05$).

Cultivar	Nodule number		Nodule weight		Dry weight	
	Large	Small	Large	Small (mg)	Roots	Shoots (g)
USG 540NRR	6	9	20.1	7.15	0.14	0.40
Progeny 5415RR	3	9	9.31	4.93	0.15	0.45
Delta Grow 5450RR	4	7	10.7	4.29	0.12	0.35
Asgrow AG5603	5	8	16.6	6.28	0.12	0.30
Asgrow AG5901	5	14	16.3	9.15	0.16	0.39
Delta King 5661RR	4	11	15.0	5.66	0.14	0.39
Delta King 5961RR	4	10	18.4	4.46	0.14	0.40
Source	DF		F-test P value			
Rep	3	0.20	0.18	0.34	0.29	0.82	0.26
Cult	6	0.72	0.20	0.59	0.27	0.01	0.01
Gly	2	0.98	0.23	0.96	0.43	0.53	0.30
Cult x Gly	12	0.43	0.62	0.31	0.51	0.35	0.14

however, for root and shoot weights, which were independent of glyphosate treatment (Table 4).

These experiments indicated that glyphosate had no effect on soybean nodulation when delivered to plants via seed imbibition. Previous research (King et al., 2001), in which glyphosate was applied foliarly to seedlings with 1 or 2 leaves, determined that glyphosate delayed nodulation and resulted in a decrease in nodule size. The difference between foliar delivery and seed imbibition of glyphosate may be due to the total amount of glyphosate delivered to roots during early stages of bacterial infection and nodulation. Seed imbibition for 3 hours generally resulted in a doubling of seed weight.

tion similarly to or greater than a foliar application at the one- to two-leaf stage (King et al., 2001). Foliar application of glyphosate had a greater effect on nodulation than did seed imbibition, however, and foliar glyphosate application would have the added benefit of decreasing weed competition in a field environment.

Decreasing nodulation by foliar glyphosate applications in glyphosate-sensitive *B. japonicum* may be one important means of increasing the specificity with which *B. japonicum* strains infect soybean. Engineering glyphosate-tolerant *B. japonicum* that had N fixation capacity greater than indigenous strains could be one means of providing this specificity and greatly increasing the amount of N required for high yields in soybean.

ACKNOWLEDGMENTS

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Juvenile justice systems:
A need for improved research and treatment

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ABSTRACT

The characteristics of juveniles who commit crimes and a variety of treatment philosophies for juvenile offenders were examined through literature and individual case studies. The literature review and three case studies provided insight into the difficult challenge of providing effective treatment programs for juvenile offenders.

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INTRODUCTION

Juvenile crime and the treatment of juveniles in the criminal justice system are an increasingly important concern in the United States. According to a 1999 national report, one in every five arrests made by law enforcement agencies involved a juvenile (U.S. Department of Justice, 1999). Juvenile delinquency is a financial and emotional drain on families and on society as a whole. It costs about $103 per day to house juvenile delinquents in detention centers in Benton County (Personal Communication, Randell Everett, Director, Benton County Juvenile Detention Center, March 15, 2002). Juvenile crime is an emotional drain because adolescents are dying, going to prison, and causing grief and suffering to families on a daily basis. Juveniles accounted for 37% of all burglary arrests in 1997, 30% of all robbery arrests, 24% of weapons arrests, 14% of murder arrests, and 14% of drug arrests. In 1997, juvenile homicides were the lowest in the decade, but still 21% above the average of adolescent homicides in the 1980's (U.S.

Department. of Justice, 1999). Although progress has been made in the area of juvenile crime and treatment, there is still a great deal of work to be done. In order to understand how to approach this issue, one must examine the characteristics of juveniles who become delinquents and the differing treatment philosophies related to adolescent rehabilitation.

What factors determine who will become a juvenile delinquent? This research project examines the characteristics of juveniles who commit crimes and a variety of treatment philosophies for juvenile offenders. It describes a local juvenile treatment facility and explores personal characteristics of a few of the adolescents sentenced to the facility. Examining these issues provides a significant challenge to social science researchers, but such research is critical if we are to change the juvenile crime statistics.

Characteristics of Juvenile Offenders

There are many people who grow up under similar circumstances but some choose delinquency and others do not. Professionals and parents would like to know

what makes the difference for these two types of individuals. Factors that contribute to delinquency include failure of adolescents to develop compassionate and empathetic feelings for others and difficulty meeting basic needs such as food, clothing, and shelter. Adolescents may turn to delinquent behaviors as they struggle to meet their emotional and physical needs (Jenkins et al., 1985).

The home life of adolescents has an influence on their involvement with delinquency. In a study to examine the influence of the family, it was found that parents of delinquents were more likely to use physical punishment than parents of non-delinquents (Conger and Miller, 1966). These researchers also found that parents of delinquents tended to express less affection, more indifference and hostility, and less warmth and sympathy toward their children. Also, compared to the control group, only a few of the juvenile offenders had close ties to their fathers. Another aspect of home life is the household structure. It was believed that a large family size increased the likelihood of becoming a delinquent because parents could not provide the proper supervision for a large number of children. Research indicates that family size alone is not a risk factor, but rather the dynamics of the family itself creates the risk. For example, if the parents or siblings are involved in criminal behavior then the likelihood increases that the juvenile will get involved with crime (Rutter et al., 1998). According to Rosenberg (1965), family structure has a major effect on adolescents. For example, research shows that, in general, children with no siblings have higher self-esteem than children with siblings. Young boys with older brothers have lower self- esteem than young boys with older sisters (Rosenberg, 1965). These findings show that family make-up has a profound impact on how people view themselves.

Another risk factor for delinquency is being from a "broken home." Research shows that delinquency is lower among adolescents who live with both biological parents than among children born out of wedlock or children from single-parent homes (Rutter et. al,1998).

According to the report by the Office of Juvenile Justice (1999), other factors that contribute to delinquency include family and individual characteristics, neighborhood environment, and daily activities. Strong demographic predictors include gender and age. Boys are much more likely than girls to become serious high rate offenders. In 2001, Benton County reported 462 intakes of males and only 93 intakes of females, and Washington County reported 478 males and only 175 females charged with criminal offenses.

Race is also a factor in juvenile delinquency. In studies of the District of Columbia and of South Carolina, it was found that African Americans were disproportionately arrested for violent crimes (Office of Juvenile Justice, 2002). The population of African Americans in South Carolina is about 30% of the total population. The study revealed that 82% of the juvenile homicide offenders referred to the solicitor were African-American.

All of the factors found to be related to delinquent behavior affect how individuals view themselves. These factors are all significant contributors to an adolescent's self-esteem, the lack of which has also been tied to delinquent behaviors.

Self-Esteem

Self-esteem is the degree of self -respect a person feels about him or herself. Self-esteem is only part of self-concept. Self-concept is how a person describes and characterizes himself or herself (Steinberg, 1996). There are many factors that contribute to a person's self-concept such as family relationships, friends, academic success, and past experiences. A person usually behaves in the manner that he or she feels represents who he or she is. This fact illustrates why it is so important to work with adolescents who are involved in the juvenile justice system to help them see themselves in a more positive light. Often juveniles in the juvenile detention center (JDC) system are referred to by labels that they then internalize. Once a juvenile is labeled delinquent, he or she often shapes his or her behavior to fit the label (Shoemaker, 1984). The juvenile then begins to experience a self-fulfilling prophecy. Often juveniles' behaviors are directly related to what they perceive others think of them or how they think of themselves (Steinberg, 1996). According to Branden (1979), people are born with the need for self-esteem but they are not born with the skills or knowledge of how to achieve self-esteem.

Research reveals that delinquents have lower self-esteem scores than adolescents who are not involved in delinquent behaviors. Ruchkins et al. (1999) tested the possible interrelationship between hopelessness, loneliness, self-esteem and personality in delinquent and non-delinquent adolescents, and found no significant difference between delinquents' levels of hopelessness or loneliness and the levels of the non-delinquent control group (Ruchkin, Eisemann, & Hagglof, 1999). However, there were significant differences in self-esteem.

Treatment Philosophies

Our society has tried many theoretical approaches to prevent juvenile delinquency but none offer a total answer. It is really going to take a wholistic approach to solve this difficult problem. One on the most common punishments for juveniles in the juvenile justice system is to be sentenced to juvenile detention, or kiddie jail.

The purpose of (JDC) is to provide a secure, safe, and caring environment for juveniles held under the authority of juvenile court (Personal Communication, Randell Everett, Director, Benton County Juvenile Detention Center, 2002). Juvenile detention centers have differing philosophies about how to fulfill this purpose.

Scared Straight. The scared straight program attempts to scare juveniles into staying out of prison. In this type of treatment, adolescents are taken on a tour of an adult prison. While there they attend an intensive confrontational session run by inmates serving long or lifetime sentences. During the session the negative aspects of prison are emphasized. The main way the inmate communicates with the juveniles is through screaming and yelling threats. Research results have shown that such programs are not effective. In fact, the approach often leads to an increase in delinquent behavior rather than a reduction (Lundman, 1984).

Incarceration. Another view of the use of the JDC is for deterrence. The supporters of this view believe that there should be more incarceration because it is a painful, appropriate consequence of a young person's involvement in delinquency. They also believe punishment of one individual will deter others from committing crimes (Lundman, 1984).

Deterrence. The deterrence philosophy has developed because research has shown that a small percentage of juvenile delinquents commit the majority of the juvenile crimes. This approach includes the concept that if repeat offenders are identified and locked up, juvenile crime rates will decrease (Lundman, 1984).

Supporters of deterrence theory view juvenile crime as an individual problem. In order to correct this problem, individuals must take responsibility for themselves. This philosophy believes that there are two steps to take in order to prevent juvenile delinquency. The first step is to identify juveniles headed for delinquency. Once the juvenile is identified, he or she is counseled by social workers, counselors, and other trained professionals to help prevent delinquent behaviors (Lundman, 1984). This philosophy sounds attractive in theory but the problem often lies in the fact that it is extremely difficult to identify juveniles headed toward delinquency. Unjustly labeling adolescents often leads to the disadvantages that come with labeling theory. However, this philosophy does influence diversion philosophy (Lundman, 1984).

Diversion. Diversion supporters believe that treating first-time offenders as if they are repeat offenders causes them to view themselves as criminals. Therefore, they express a self-fulfilling prophecy and become serious offenders (Lundman, 1984). Missouri, Tennessee, Florida, and New York participated in a national evaluation of diversion projects. In the evaluation, juveniles were referred by police and prosecutors. These juveniles received individual and family counseling along with employment, educational, and recreational services. After examining all the research, it was concluded that diversion should be the first option for juveniles that commit status or minor offenses (Lundman, 1984). Status offenses are offenses that are only illegal because of the age of the offender, for example, truancy and under-age drinking (Steinberg, 1996).

A Case Study

Washington County Regional JDC provided an opportunity for a case study of a facility that believes in the integrated model of adolescent rehabilitation. Although the Washington County Regional JDC is officially just a "detention center," it has many characteristics of a diversion program. Washington County is experiencing a rapid and dramatic population growth. As the overall population grows, crime rates also tend to increase. Washington County Regional JDC has 36 beds, which are usually full. The primary reason juveniles are sentenced to the JDC is probation violations. Therefore, it is important to establish JDC programs that effectively reduce recidivism rates among juveniles.

The Washington County JDC implemented two new programs in 2002. The first program is a computer skills program called Tech Life, designed to teach adolescents skills that will help them when they are released. In addition, it is believed that becoming competent in computer skills will increase their overall sense of competency. The self-esteem element of the program is a by-product of developing that competency. As the computer skills help the youth get good jobs, their self-esteem increases because they see and experience more options for their life outside the criminal justice system.

The second program is entitled BARK. This program is designed to teach the adolescents to take responsibility for animals and realize their sense of self worth by doing so. In the program, dogs from the local animal shelter are brought to the JDC where the resident adolescents will the dogs to be helpers for families with disabled people. The youth also are involved in showing the family proper pet care so the resident adolescents must learn how to be responsible for another living creature. The staff at the JDC believes that taking responsibility for another creature and receiving the unconditional love animals often give helps individuals of all ages develop a stronger sense of competency and a higher level of self-esteem.

The center also has long-standing programs such as counseling and educational programs. These collabora-

tive programs are with Youth Bridge, Ozark Guidance Center, Fayetteville Public Schools and other family services providers.

The self-esteem scores for juvenile delinquents are on average lower than those of their non-delinquent peers. One of the goals of this study was to explore whether or not adolescents in the Washington County Regional JDC scored as high as non-delinquent youth in other research studies. However, because of the small sample size, that comparison was not possible. It proved to be more practical and beneficial to do individual case studies.

MATERIALS AND METHODS

Sample

The sample for this study consisted of juvenile offenders sentenced to the Washington County Regional JDC. All necessary federal reviews for the protection of human subjects were completed. Signed consent forms were completed by both the adolescents and the parents. Only those adolescents who complete parental consent forms were allowed to be considered for participation in this research project. If a visiting parent signed the consent form, their adolescent was asked to participate and sign a consent form of his or her own. Questionnaires were then distributed to juveniles who signed the required form.

Measures

The participating juveniles completed Rosenberg's self-esteem survey. They also answered questions about their personal and family characteristics, family life, experiences at the JDC, and their interactions with the JDC staff.

Rosenberg Self-Esteem Scale. The Rosenberg Self-Esteem Scale (Rosenberg, 1965) is one of the most widely used self-esteem measures in social science research. There are 10 questions using a four-point scale. Responses vary from 1 = strongly disagree to 4 = strongly agree. Items numbered three, five, eight, nine, and ten are reverse-coded for analysis purposes. Previous research indicates that the Rosenberg Self-Esteem Scale has acceptable reliability for this type of study (DuBois, 1996).

Other Questionnaire Items

The questionnaire also included questions about demographic information, attitudes, and the JDC program. The complete questionnaire is attached as Table 1.

Results

Because of the variation in length of court sentencing and the transitory nature of being sentenced to the JDC, the population pool was very small. The size of the population was reduced because opportunity for parental consent was limited to weekend visiting days. Also, many of the parents did not come to visit the adolescents on the visiting days. Once consent forms were gathered from both the parents and the juveniles, the actual number of respondents participating in the study was only three. Obviously, this number is too small to provide representative statistical analysis. However, the data from these individuals provides some information about a very select group of juvenile offenders sentenced to the JDC. The results reflect individual case studies. All identifying personal information has been omitted from this report.

The participants ranged in age from 15-17 years old. None of the participants came from a household with two biological parents. The structure was either a single parent household or a household including a step-parent. All of the participants had siblings. According to respondents none of these factors contributed to the participants becoming involved with delinquency. However, as other research suggests, these factors may have influenced the choices that they made. In the discussion of the results participants will be referred to as X, Y, and Z and male in gender.

According to the self-report responses, participant X felt that the JDC staff cared. Participants Y and Z felt that the staff was neutral in regard to caring for the participants. Participant X felt that the JDC had a negative effect on him. Participant Y did not feel that the JDC had a positive or negative influence. Participant Z felt that the JDC had a very positive influence. Participants X and Y were neutral in rating the staff interaction. Participant Z felt that there was a great deal of staff interaction. All of the participants felt they had good family lives. Each participant saw the importance of an education. Participant Y felt better about himself than when he entered the JDC. Participants X and Z did not feel better about themselves after being in the JDC. No participant felt worse about himself after being in the JDC. Each participant saw a need for change in his life. Participant X felt that his parents had a negative influence. Participant Y felt that his parents did not influence him negatively or positively. Participant Z felt that his parents influenced him positively. Participant X felt that he learned techniques that would keep him away from future criminal behavior. Participant Y felt he learned techniques that would keep him out of trouble. Participant Z felt that he learned techniques that would keep him out of trouble and keep him from returning to Washington County Regional JDC.

The self-esteem scores ranged from 21 to 35. The highest score possible was a 40. Individual scores were; X 21, Y 24, and Z 35.

RESULTS AND DISCUSSION

With only three participants, the survey provided no useable data, but the attempted study provided an opportunity to get a closer look at the juvenile justice system and the juveniles it serves. Each question had an opposite item on the questionnaire. On several of the questions the participants answered with two different opinions. These seemingly conflicting responses could be explained by the fact that the participant could have been thinking about different staff members or experiences when answering each question. It is also very important to note that it is difficult for the JDC to substantially influence the participant, even though there are several different programs offered by the Center, because of the limited time adolescents spend in the Center.

When examining the results, it is interesting to note that the participant with the highest self-esteem score felt that the JDC had a positive influence. The participant who felt JDC had a negative effect on him had decided to give up the life of delinquency but was arrested just before this change of mind. This response implies that if a person is ready to give up crime, placing him in a facility that punishes of criminal behavior by lock-up with other offenders could actually reinforce negative influences.

The participant who scored very high on the self-esteem scale and felt like he had a good family life did not fit the typical characteristics of juvenile delinquents. This demonstrates the fact that there is likely no way to identify all adolescents headed for delinquency.

In order to make a difference in juvenile crime rates, society should begin to monitor juvenile offenders. Since offender records are limited, one cannot determine which programs are effective and which are ineffective for reducing juvenile crime. There are many different ways to approach preventing and treating juvenile delinquency. Until we examine the strengths of various approaches and create a treatment that encompasses the strengths of productive philosophies we will continue to hear about soaring juvenile crime rates.

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Table 1. Questionnaire

Please respond to the following questions to the best of your knowledge. Check the response that best fits.

Age   ___ 10 _____11 _____12 _____ 13 _____14 _____15 _____16 _____17 _____18

Gender: _____male   _____female

Grade level currently In _____

Parents' education level:
Mother: _____ middle school/ junior high, _____some high school, _____high school diploma/GED, _____ some college, _____ community college/technical school, _____ college degree or beyond, _____don't know.

Father: _____ middle school/ junior high, _____some high school,_____high school diploma/GED, _____ some college, _____ community college/technical school, _____ college degree or beyond, _____don't know.

Family household structure
(1)   One parent living in household _____
(2)   Both Parents living in household _____
(3)   One biological parent and one step parent in household _____
(4)   Live with other relatives _____
                If yes, Who?
                        a. Brother or sister _____
                        b. Grandparent _____
                        c. Other relative_____
(5)   Live with friends _____

Number of Brothers _____ and/or _____ Sisters

List all the programs you have participated in at the JDC?
            --Specific programs will be listed on the blackboard –

1. _____

2. _____

3. _____

Which program do you like the most?
            Why?

Which do you like the least?
            Why?

Is this your first offense? Y or N

What was the offense that led to your time in the JDC?
_____

_____

Table 1. Questionnaire, continued

Answer the following questions on a scale of 1 – 4 (1 = Strongly disagree, 2 = disagree, 3 = agree, 4 = strongly agree)

I feel the staff here really care about me and my well being.	1	2	3	4
JDC has had a positive influence on me.	1	2	3	4
There is very little staff interaction here.	1	2	3	4
I feel like I had a bad family life.	1	2	3	4
I see no reason to be concerned about my education.	1	2	3	4
I feel better about myself now than when I first came.	1	2	3	4
I see no need for change in my life.	1	2	3	4
I feel worse about myself now than when I first came.	1	2	3	4
My parent(s) influence me positively.	1	2	3	4
JDC has had a negative impact on me.	1	2	3	4
I feel the staff really do not care about me or my well being.	1	2	3	4
I learned techniques that will help keep me out of trouble.	1	2	3	4
There is a great deal of staff interaction.	1	2	3	4
I now see the importance of my education.	1	2	3	4
My parent(s) influence me negatively.	1	2	3	4
I feel like I have a good family life.	1	2	3	4
I see a need for change in my life.	1	2	3	4
I learned techniques that will help keep me from unlawful behavior.	1	2	3	4

Please respond to each of the following statements with the response that best describes your feelings about yourself. Please place the number of the response that best describes you on the line to the left of the question.

Respond according to the following scale:

(1) Strongly disagree
(2) Disagree
(3) Agree
(4) Strongly agree

_____ 1. I feel I am a person of worth, at least on an equal basis with others.

_____ 2. I feel that I have a number of good qualities.

_____ 3. All in all, I am inclined to feel that I am a failure.

_____ 4. I am able to do things as well as most other people.

_____ 5. I feel I do not have much to be proud of.

_____ 6. I take a positive attitude toward myself.

_____ 7. On the whole, I am satisfied with myself.

_____ 8. I wish I could have more respect for myself.

_____ 9. I certainly feel useless at times.

_____ 10. At times I think I am no good at all.

Is there anything else you would like to say?

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

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

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

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