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DISCOVERY

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Cover: Arkansas rice and soybean fields (2003 cropping season) mature in the historic landscape of surviving farmhouses from the Roosevelt-era Plum Bayou Resettlement Community, built as a model solution to tenant and sharecropper land-tenure grievances in Arkansas during the Great Depression. Photo courtesy of John Crone, Professor, Department of Landscape Architecture, School of Architecture, University of Arkansas.

Letter from the Dean

Gregory J. Weidemann

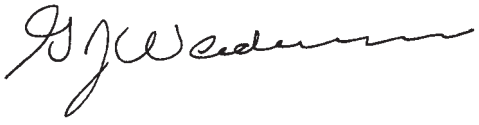
Bumpers College of Agricultural, Food and Life Sciences provides many opportunities for undergraduate students to engage in learning experiences beyond the normal course work. Conducting a research project or creative project with a faculty mentor is one of these opportunities. Sixty faculty members presently serve as mentors to students to assist them with research and creative projects. Most, but not all, such projects are designed to meet the requirements of an honors thesis in the Bumpers College Honors Program. Whether in the Honors Program or not, students who take advantage of such opportunities find that it brings into sharper focus principles they learn in class. It is truly an added value in their university education.

The Bumpers College encourages student research by awarding undergraduate research grants, including the Carroll Walls Undergraduate Research Fellowship, which provides a grant of $1,000. We awarded 22 Undergraduate Research Grants in the fall of 2002.

Discovery provides a reporting outlet for our student scientists, which allows them to bring the research process to fruition. It does not supersede publication elsewhere, but it does provide a forum for students and faculty to share their results and findings in a citable publication.

The 15 articles in this fourth annual volume of *Discovery* are on a wide range of topics. They include production issues in rice, organic apples, broiler chickens, and blackberries. Topics also include environmental issues and functional genomics research. We are proud to present these articles as examples of the research accomplishments of our undergraduate students.

I heartily congratulate the student authors on their accomplishments and extend thanks to their faculty mentors and to the editors who reviewed their manuscripts. Thanks also to the Honors Committee for providing a structured program that encourages our students to spread their wings in this way.



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

The characteristics of consumers and producers using farmers' markets

Walter H. Añez[*] *and Michael R. Thomsen*[†]

ABSTRACT

This study examines the perceptions of both consumers and producers towards farmers' markets. Consumer perceptions are generalized from several previous studies conducted throughout the United States. Producer information was gathered through a survey of members of one farmers' market in Arkansas. Previous studies, conducted in both rural and urban areas, indicate that the typical famers' market customer is well educated and is of higher than average income. These consumers place great importance on quality of produce, knowledge that produce is grown locally, and the social interaction obtained through the farmers' market experience. For producers, the farmers' market is an important outlet for produce and in many cases accounts for all of their produce sales. The survey results suggest that there is an interest in expansion on the part of many producers and that additional acreage is available for growing produce crops. In addition to providing a market outlet, producers also benefit from the social dimension of the market.

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† Michael R. Thomsen, faculty sponsor, is an assistant professor in the Department of Agricultural Economics and Agribusiness.

MEET THE STUDENT-AUTHOR

Walter H. Añez

After two years in a private university in Santa Cruz, Bolivia, I came across the opportunity to attend the University of Arkansas thanks to a State of Arkansas-Bolivia partnership agreement. I chose the agricultural business and economics undergraduate program, a program that gave me the tools I needed to conduct research and expand my perspective in the study of agricultural economics.

After the first year at the U of A, I started working with Dr. Michael Thomsen. I first worked on a market case study for a private company in Fayetteville, after which I was hired by the company for an 8-month internship that I completed during the fall and spring semesters of my senior year. During the internship, I had the opportunity to conduct economic research on a variety of issues in several different agricultural industries, both in the U.S. and worldwide.

The second project I became involved in was the one that motivated this paper. This project has allowed me to study another aspect of economics, that being the economics of small farmers.

The practical experience I have gained while working with Dr. Thomsen and on the internship has allowed me to use the knowledge obtained in the classroom and apply it to real world situations. These experiences will be invaluable when I begin the M.S. program in agricultural economics at the University of Arkansas in Fall 2003. I thank Dr. Thomsen for these opportunities, and for his support and encouragement.

INTRODUCTION

Increasingly, small farms are relying on direct-marketing options as an outlet for their horticultural crops. Direct marketing encompasses several venues, the most common being roadside stands, direct-farm markets, pick-your-own operations (PYO), and farmers' markets. Roadside stands are usually temporary structures erected by farmers for the purpose of selling seasonal farm produce. In PYO operations, consumers harvest the produce from the farmer's field. On-farm markets are permanent structures located at the farm gate to sell the farm's own produce. Farmers' markets are operations where farmers transport their products to consumers. Among the most popular items sold through such direct-marketing venues are fruits, vegetables, flowers, nursery products, eggs, and dairy products (Govindasamy and Nayga, 1997).

Direct marketing has become increasingly important for small farms over the past several years. Improvements in transportation, refrigeration, and communication technology have consolidated marketing outlets within the retail food industry and have changed the fresh-fruit and vegetables industry from regional markets to a national market. One advantage of this is that a wider variety of fresh fruits and vegetables are available to the population as a whole (Fjeld and Sommer, 1982). However, these changes have created both economic and non-economic complications for small farms. Large farmers have been able to set the pace regarding standardization of products and better supply-management practices. Moreover, marketing firms have turned to large farms in order to be able to deliver products throughout the U.S. and abroad. This has created a complex marketing system that makes it difficult for small farmers to compete because they often lack the technical efficiency, access to capital, and other vital components that are more readily available to larger producers (Epperson and Estes, 1999; Gebremedhin and Christy, 1996).

In response to these changes in the marketing structure, small farms are increasingly relying on direct markets as an outlet for their produce. At the national level, the number of farmers' markets has grown by 79 percent during the past seven years and farmers' markets are the

only market outlet for 19,000 farms (USDA Agricultural Marketing Service, 2002). Direct-marketing outlets are of even more importance in states such as Arkansas given the predominance of small, family-owned operations. Detailed information about the importance of direct marketing regionally is unavailable; however, a study of fruit and vegetable growers in Oklahoma conducted in the late 1980s found that nearly 90% used some form of direct marketing (Henneberry and Willoghby, 1989).

The growing role for direct-marketing outlets suggests that there are some consumer preferences that are not being met by the complex marketing chain (retail grocery stores) that has emerged for produce crops. Over the years, several studies have addressed the characteristics of direct-marketing outlets that consumers perceive to be important. Equally important is an understanding of the characteristics of small farmers that use them. This type of information is useful for those involved in rural development policy and extension work in addition to those more directly involved in direct marketing.

In this study, the focus is specifically on farmers' markets. The research objectives are twofold:

(1) To summarize information about the characteristics and preferences of consumers who use direct-marketing facilities, with a particular emphasis on farmers' markets;

(2) To gather information about the characteristics of producers selling through a farmers' market in Arkansas.

MATERIALS AND METHODS

Over the past two decades, several studies were conducted that examined the characteristics of consumers who visit farmers' markets and the aspects of the market that they perceive to be of value. Objective 1 was accomplished by a review of these studies and generalization of main findings about farmers' market consumers. These studies examined farmers' markets in Maine (Buitenhuys et al., 1983; Kezis et al., 1998); Tennessee (Eastwood et al., 1999); New Jersey (Govindasamy and Nayga, 1997); Delaware (Gallons et al., 1997); and California (Fjeld and Sommer, 1982). The location of the studies provides geographically diverse information about consumers, and generalizations can be made from the findings. Moreover, several of the studies address markets in rural as well as metropolitan areas, giving a broader sample of people from different socioeconomic environments. For instance, Eastwood et al. (1999) examined six farmers' markets located throughout Tennessee. Also, Gallons' et al. (1997) study involved surveys sent to 10,000 consumers and represented all of the counties in Delaware.

Very few studies address the characteristics of producers that sell through farmers' markets. The only study to date was conducted over 20 years ago (Brooker and Taylor, 1977). Hence, to accomplish Objective 2, a survey of producers selling through a farmers' market in the city of Fayetteville, Ark. was conducted. The survey instrument was distributed to producers at a membership meeting in fall 2002. The main goals of this survey were to collect information on the members' motivations to participate in the farmers' market and the importance of the local farmers' market as an outlet for fruits and vegetables. The survey also elicited information on member interest in expansion of production activities. Twenty-seven surveys were completed by producers at the membership meeting; only producers that sold crop produce were asked to complete the survey.

RESULTS AND DISCUSSION

Consumer Characteristics

In all of the studies reviewed descriptive statistics were provided; these were analyzed and inferences were drawn about the typical characteristics of consumers at farmers' markets. The goals stated in the six studies were focused on how to improve services at farmers' markets and make better promotion decisions. Some of the goals were to:

- Determine consumer perception of, expectations of, and preferences for direct markets.
- Determine consumer socioeconomic characteristics and their influence on purchasing habits, attendance at direct-marketing outlets, and patronage trends.
- Evaluate consumer's recognition of in-state produce.
- Study the effects of regional produce characteristics on consumers' eating habits.

Eastwood et al. (1999) found that the typical shopper at Tennessee farmers' markets is a white female who is over 45 years of age, has above average income, and high education level. Gallons et al. (1997) indicated that the typical shopper at Delaware farmers' markets had almost exactly the same characteristics. Similar characteristics were also found in the 1981 and 1995 surveys conducted in Maine (Buitenhuys et. al., 1983; Kezis et al., 1998). Govindasamy and Nayga (1997) used a logit analysis to estimate the likelihood that people with certain socioeconomic characteristics would visit a farmers' market. They found that higher-income, higher-educated shoppers were more likely to visit and spend more money than people with other socioeconomic characteristics. Fjeld and Sommer (1982) noted similar results in their survey of California consumers.

When comparing the survey data from the six consumer studies, it appeared that shoppers at farmers' markets, regardless of location, had the same general charac-

teristics including greater-than-average income levels and higher education levels. This conclusion is corroborated by Kezis et al. (1998) who stated that the higher education and income levels were found "…among farmers' market patrons that have been consistently noted in most studies of other markets conducted over the years" (Kezis, et al., 1998).

Consumer Perceptions

The studies reviewed also provide information about factors that drive frequency of visits and aspects of farmers' markets that consumers perceive to be of importance. Eastwood et al. (1999) found that shoppers who place more importance on (a) quality selection, (b) desire to help local farmers, (c) nutrition, (d) freshness, (e) knowledge that produce is locally grown, and (f) atmosphere, were more frequent visitors to farmers' markets. Descriptive statistics drawn from Gallons et al. (1997) suggest that most shoppers perceive (a) produce selection, (b) knowledge that produce is locally grown, and (c) helping local farmers as the most important reasons for purchasing at farmers' markets. In their study a greater percentage of shoppers (65%) visited the market with family members; although not stated in the study, this suggests that people perceive shopping at farmers' markets as a social recreational activity.

Kezis et al. (1998) provided similar evidence on consumer attitudes toward farmers' markets. In their study, quality of produce was selected as a key attraction, followed by supporting local farmers and a friendly atmosphere. Furthermore, the 1981 Maine survey presented largely the same consumer perceptions about farmers' markets (Buitenhuys et al., 1983).

According to the responses given by consumers at direct-marketing outlets, they expect to find good-quality produce, wide variety, fresh fruits and vegetables, and a friendly atmosphere. These were some of the reasons these consumers gave for visiting the farmers' market.

Producer Characteristics

In evaluating the importance that producers placed on reasons for participation in an Arkansas farmers' market, the average of the importance ratings fell between (3) important, and (4) very important. The one exception -- that farmers' market participation is a good family activity -- rated at 2.8 on average, just below important (Table 1). One implication of these results is that non-economic reasons -- i.e., providing the community with access to locally grown products -- are considered to be very important by many producers who use the market.

Those answering the participation frequency survey were quite active in the market with the majority (58%) participating in the market throughout all or most of the season (Table 2). Furthermore, 69% of the respondents attended the market more than once each week during the portion of the season they were active in the market. The survey requested information about (1) the acreage that was suitable for cultivation of produce crops, and (2) acreage that was currently in use to produce these crops. Twenty-five respondents answered these questions. The responses indicated that on average there are 9.8 acres per operation that were currently suitable for cultivation of fruits, vegetables, or other produce crops. On average, 3.6 acres were currently in production. Thus, if one were to consider the hypothetical case of one large operation comprising the acreage of all respondents, then (3.6∏9.8)*100=37% of available land to producers was being used to grow produce crops.

Another way to examine the responses is to see what percentage of available land on average is being used to grow produce crops. If AP = acreage currently in use to grow produce crops and AT = total acres suitable for production of these crops, the average ratio of AP∏AT was 0.60 indicating that, on average, respondents were using 60% of available land for production of produce crops.

The calculation of this 60% value weights each respondent equally regardless of size. A weighted average of the ratio AP∏AT that gives more influence to operations with large available acres was only 26%.[1] The difference between the simple average number of 60% and the weighted average of 26% suggests that land use percentages were substantially lower on the larger acreage operations.

It should be noted that available land not used to produce fruit and vegetable crops could have already been in use for non-edible crops that were sold through the market; i.e., flowers. Also, land may have been generating a return through an alternative use. However, responses (n=26) indicated, that over 60% of respondents had a strong interest in expansion, and 93% indicated they were at least somewhat interested (data not shown).

The importance of farmers' market revenue in total family income varied considerably among respondents (Table 3). The farmers' market accounted for the vast majority of revenues (over 80%) generated from the sale of fruit and vegetable crops by producers (Table 4).

Opinions on the seriousness of several fresh-market problems indicated that on average, respondents rated each problem somewhere between a minor problem and a somewhat serious problem (Table 5). A concern that existing market outlets were inadequate was the only

[1]In this case the weight used for a respondent is the acreage suitable for produce crops divided by the sum of suitable acreages from all respondents.

problem that received a somewhat serious to serious rating from the majority.

The highest-frequency response to disposition of produce that was not sold was giving excess produce away or preserving it for home use (Table 6). One interpretation of these responses is that respondents have fairly good forecasts of what can be sold through the farmers' market and thus grow produce in quantities adequate to meet but not exceed this demand.

From the results of both the consumer studies and the producer survey, one conclusion is that farmers' markets are of great importance to both consumers and producers. From the consumer side, farmers' markets provide social interaction with farmers and access to products perceived to be of high quality; thus, consumers place relatively low importance on price. From the producers' side, farmers' markets are important outlets for produce, with the survey results suggesting that farmers' markets account for most and often all of total produce sales. Furthermore, farmers' markets sales can be important to producers' household income.

From a social standpoint, farmers' market operations are promoters of social interaction among buyers and sellers -- an interaction that is not experienced by shoppers at "super-centers" or "supermarkets" (Hinrichs, 2000). The studies reviewed to reach Objective 1 in this paper support Hinrichs' assertion regarding the social benefits provided to consumers by farmers' markets. Many of the positive consumer perceptions involve not only benefits that concern exchange of commodities, but also those related to social interaction caused by the congregation and association of producers and consumers. Producers also rated non-economic benefits highly. This shows the importance of peripheral benefits to both consumers and producers. These preferences provide farmers' markets with advantages over other retail outlets and can be used to develop potential marketing ideas that can improve profits in farmers' markets in Arkansas.

ACKNOWLEDGMENTS

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Table 1. Producer reasons and importance for selling at an Arkansas farmers' market.

Reason	(1)Not important %	(2)Somewhat important %	(3)Important %	(4)Very important %	N	Mean
Interaction with customers and other vendors	0	8	46	46	26	3.4
Stay involved in agriculture	0	4	40	56	25	3.5
A good family activity	8	23	46	23	26	2.8
Provide community access to locally grown produce items	0	4	23	73	26	3.7
Rely on income	0	20	32	48	25	3.3
Limited access to other market outlets	8	19	19	54	26	3.2

N = Number of responses

Table 2. Producer frequency of selling at the farmers' market (N = 26).

Frequency	Percent of respondents
Once a week through all or most of the market season	8
More than once a week through all or most of the market season	50
Once a week for a portion of the market season	23
More than once a week for a portion of the market season	19

Table 3. Percent of total household income derived through farmers' market sales (N = 26).

Response	Percentage of respondents
Less than 5 percent	35
5 to 15 percent	15
16 to 30 percent	15
31 to 50 percent	12
More than 50 percent	23

Table 4. Market outlets used to sell produce crops, average percent of revenues (N = 23).

Market outlet	Percent of revenues
Farmers' market	81.3
Other direct marketing	0.0
Wholesalers	4.8
Retailers (grocery stores)	4.2
Restaurants or other food service	5.0
Other market outlets	4.8

Table 5. Importance of marketing problems for farmers' market producers.

Problem	(1)Not a problem %	(2)A minor problem %	(3)A somewhat serious problem %	(4)A very serious problem %	N	Mean
Existing market outlets are inadequate	33	13	33	21	24	2.4
No outlet for produce cosmetically unsuitable for fresh market	23	41	23	14	22	2.3
Surplus produce is wasted or salvaged at low value	28	28	28	16	25	2.3

N = Number of responses

Table 6. Disposition of produce that doesn't sell at farmers' market.

Disposition	(1)Almost never %	(2)Infrequent %	(3)Sometimes %	(4)Often %	(5)Almost always %	N	Mean
Discarded	27	23	36	5	9	22	2.5
Preserved for own use	0	32	36	27	5	22	3.0
Given it away	8	17	25	42	8	24	3.3
Sell elsewhere	33	22	6	33	6	18	2.6

N = Number of responses

Propagation of thornless Arkansas blackberries by hardwood cuttings

Mark M. Bray[*]*, Curt R. Rom*[†]*, and John R. Clark*[§]

ABSTRACT

Effects of auxin application and cutting location on canes on adventitious root development in hardwood cuttings of three Arkansas thornless blackberry cultivars were studied. Dormant canes were collected from one-year-old plants of 'Apache', 'Arapaho', and 'Navaho' and stored in a cold room until February. Two- or three-node cuttings were taken from the canes at apical, mid, and basal locations along the cane and were placed under intermittent mist in a perlite-filled greenhouse bed. Cuttings were either untreated or treated with auxin indole-3-butyric acid (IBA), applied as a liquid quick dip at 0.3%. In general, cutting diameter was greatest for basal and smallest for apical cuttings. Significant interactions were observed for cultivar and cutting location and for cultivar and auxin treatment for rooting. 'Apache' with auxin treatment had the highest rooting percentage, and 'Arapaho' and 'Navaho' with auxin the lowest. For cuttings that rooted, auxin treatment increased the root rating, representing root system development, for 'Apache' and 'Navaho' but had no affect on 'Arapaho'.

[*] Mark Bray is a senior majoring in horticulture.

[†] Curt R. Rom, faculty sponsor, is an associate professor in the Department of Horticulture.

[§] John R. Clark, faculty sponsor, is a professor in the Department of Horticulture.

MEET THE STUDENT-AUTHOR

Mark M. Bray

After graduating from Benton High School in 1992, I attended the University of Arkansas at Fayetteville where I majored in botany. While studying at the University of Arkansas, I worked as a research assistant for the Botany Department. I worked on a census project where I counted species of rare, threatened, and endangered wildflowers along the Buffalo National River. During this time, I also volunteered my time to the Ozark Natural Science Center. In 2001, I returned to the University of Arkansas to pursue a degree in horticulture. Since then, I have been actively involved with the Horticulture Club.

While pursuing my B.S. degree in horticulture, I received the Northwest Arkansas Tomato Growers Association Scholarship. During the summer of 2003, I worked as an Adair Scholar in the Plant Pathology Department at the University of Arkansas. My project was to identify and characterize a new plant virus affecting blackberries. I began graduate school in fall 2003 at the University of Arkansas. Before my senior year as a horticulture student, Dr. Rom and Dr. Clark suggested the idea for my undergraduate research project. Because of my interest in small fruits, the opportunity to study propagation methods for blackberries appealed to me. Since propagation guidelines have not been established for Arkansas thornless blackberries, the research project has the potential to provide useful information for the nursery industry.

INTRODUCTION

Advances in blackberry cultivars from the University of Arkansas fruit breeding program have contributed significantly to the expansion of commercial blackberry production (Clark, 1999). Thornless blackberry cultivars released from the University of Arkanasas breeding program that have contributed to this expansion include 'Apache' (Clark and Moore, 1999), 'Arapaho' (Moore and Clark, 1993), and 'Navaho' (Moore and Clark, 1989).

With this expansion the demand for plants has increased along with a need for improved methods of propagation (Busby and Himelrick, 1999). Nurseries have utilized various propagation techniques and types of vegetative material to propagate desirable genotypes. Common methods of propagating blackberries include: tip layering, leaf-bud cuttings, tissue culture, and root cuttings (Caldwell, 1984). Each method has its disadvantages. Tip-layering involves excessive hand labor to separate canes, few propagules are produced per plant, and weeds are difficult to manage. Successful propagation by tip-layering is also difficult to achieve with erect-cane genotypes. The leaf-bud softwood cutting method has been utilized for high production with some success. Leaf-bud location along cane was observed to affect rooting; consequently, this method has not been widely adopted (Caldwell, 1984). The disadvantage of the tissue culture method is the large initial investment of high-tech expensive propagation equipment. Therefore, the use of this method may not be appropriate for small nurseries. Indubitably, growers will choose the method of propagation that most greatly reduces production costs (Caldwell, 1984).

Hardwood cutting propagation is not used commercially but might be of value since the cane material utilized with this method is traditionally pruned and discarded. The advantages of propagating by hardwood cuttings would be the production of more propagules per plant, the utilization of excess cane material, and a possible reduction of the spread of virus infections. Propagation techniques that generate an increased number of propagules per plant, from otherwise useless, excess plant material would reduce propagation costs for nurseries and could reduce plant costs for growers. In addition to reduced costs, another benefit for growers would be a possible solution to virus transmission

among plants. By mass propagating from virus-indexed stock, growers would reduce the rate at which virus infections are spread (Ahrens, 1991). Furthermore, this method of propagation could be useful to plant breeding for timely pollen collection or manipulated pollination procedures (Lopez-Medina and Moore, 1997).

Propagation of blackberries by hardwood and softwood stem cuttings has been successfully achieved without the use of auxin rooting hormone. However, the application of auxin when propagating *Rubus* sp. by stem cuttings has proved valuable for root system enhancement (Busby and Himelrick, 1999; Williams and Norton, 1959). Busby and Himelrick (1999) reported that root development increased on 'Navaho' softwood cuttings treated with 0.3 and 0.8% K-IBA quick dip. Lopez-Medina and Moore (1997) reported that the application of 0.3% IBA improved the volume of roots formed in Arkansas erect-cane blackberries propagated by floricane dormant stem cuttings. Our experiment further studied the propagation of thornless erect-cane blackberries by dormant hardwood cuttings. The purpose was to evaluate a mass propagation system that is simple and efficient for commercial production of several thornless Arkansas blackberries. Specific objectives were to evaluate the effects of cultivar, auxin, and cutting location on rooting of hardwood cuttings.

MATERIALS AND METHODS

On 9 Dec. 2002, at the University of Arkansas Agricultural Research and Extension Center, Fayetteville, one-year-old canes of 'Apache', 'Arapaho', and 'Navaho' were removed from plants grown in cold-frame growing beds and placed in sealed plastic bags. The canes were placed in cold storage at 7°C for approximately two months to satisfy chill requirement. Two- to three-node hardwood cuttings (10-12 cm long) were taken on 12 Feb. 2003. The cuttings were taken from three locations on the cane: apical, mid, or basal sections. The basal cut on each cutting was made at approximately 1.5 cm below the basal node. Cuttings were treated with either no hormone treatment (control) or treated with rooting hormone. The rooting hormone treatment consisted of a commercial auxin (0.3% IBA solution) with a quick dip of 1-3 s. Cuttings were immediately inserted approximately 5 cm deep into propagation beds consisting of a perlite rooting medium. The propagation beds were equipped with heating cables for bottom-heat at 22-25°C, and a mist system. The automatic intermittent mist system was set to mist for approximately 16 s every 10 min during daylight hours. Cuttings remained under mist for 45 d, and then were removed for evaluation of root systems. Propagation beds were located in a heated greenhouse with daily minimum temperature of 15°C and a daily maximum of 25°C. No supplemental lighting was provided. Rooted cuttings were potted in soilless medium for plant establishment.

The experimental design was a three by two factoral design (three cultivars and two auxin treatments) arranged in a randomized complete block design with seven replications of three cuttings for each cultivar and auxin treatment. Treatments were blocked by location in mist beds. Data collected were cutting diameter, number of cuttings that rooted, and rooting rating. The number of cuttings that rooted was expressed as percentage of rooted cuttings. The rating system was a subjective rating of 0 to 5 where 0 = the absence of roots or a dead cutting, and 5 = an extensive root system. Data were analyzed by analysis of variance using Statistical Analysis System (SAS), and means separated by t-test.

RESULTS AND DISCUSSION

Analysis of cutting diameter indicated significant ($P< 0.05$) sources of variation were cutting location on cane, cultivar and the cultivar by location interaction. For rooting percentage, significant effects were auxin treatment and the cultivar by auxin treatment interaction. Rooting rating sources of variation that were significant were cultivar, auxin, and the cultivar by auxin interaction. Due to significant interactions of all variables, the interaction means will be presented.

Average diameters for apical cuttings of all three cultivars were similar (Table 1). Cuttings taken from mid sections of canes for 'Apache' and 'Navaho' were similar. However, mid-section cuttings of 'Arapaho' averaged 0.8 mm larger in diameter than 'Apache' mid-section cuttings, and 0.6 mm larger in diameter than for 'Navaho'. Furthermore, the average diameters of basal cuttings for all three cultivars were different. 'Arapaho' cuttings were the largest, averaging 0.8 mm larger in diameter than 'Apache', and 0.4 mm larger than 'Navaho'. Although there were some significant differences for cutting diameter, we thought the diameter differences for the cuttings would be negligible for potential rooting. This was confirmed by a Pearson Product Moment Correlation that was conducted on the cutting diameter and rooting percent data. The results of the correlation were not significant ($P< 0.05$) and the r value = 0.15. These findings indicate that cutting diameter had no relationship to rooting in our study.

Significant auxin-by-cultivar interaction for rooting percentage was observed (Table 2). Within the no-auxin treatment, there were no significant differences among

cultivars in the control. However, within the auxin-applied treatment, there were significant differences among cultivars. Percent rooting for 'Arapaho' was greatly reduced (47.5% to 14.2%) with the application of auxin. The cultivar with the highest percent rooting was 'Apache' with auxin treatment (47.6%). However, auxin treatment reduced rooting percentage for 'Arapaho' and 'Navaho' compared to control. These findings indicate that with the auxin concentration used (0.3%), these three cultivars responded differently to auxin treatment. For cuttings that rooted, root rating had significant differences for cultivar and auxin treatment (Table 2). Root ratings for 'Apache' and 'Navaho' with auxin treatment were greater compared to non-treated control. 'Arapaho' cuttings that rooted showed no difference in rooting rating with auxin treatment compared to control.

Several studies on the propagation of blackberries by stem cuttings have been reported on with inconsistent performance records. Bobrowski, et al. (1996) reported that propagation by hardwood stem cuttings of blackberries is simple but rooting is not always satisfactory. Similarly, we observed that less than 50% of hardwood cuttings established roots. Zimmerman, et al. (1980) reported that node position on the cane had no significant effect on rooting of 1-node softwood cuttings, and that softwood cuttings rooted better than one-node, and much better than three-node, hardwood cuttings for cultivars 'Smoothstem' and 'Dirksen Thornless' blackberries. Zimmerman also reported that IBA had little effect on rooting of softwood and hardwood cuttings for cultivars 'Smoothstem' and 'Black Satin'. Lopez-Medina and Moore (1997) reported that significant differences in percentage of cuttings rooted occurred only for 'Arapaho' and 'Shawnee' but not 'Navaho'. In contrast with Zimmerman's findings, Lopez-Medina and Moore also reported that cultivar by position interaction effect was evident and IBA improved volume of roots formed (Lopez-Medina and Moore, 1997). Busby and Himelrick (1999) reported that thornless blackberry cultivars rooted easily from softwood cuttings in mist beds without the application of auxin, but that with auxin (0.3% and 0.8% IBA) root system development was enhanced for 'Navaho'. However, no specific auxin concentration consistently improved rooting for the cultivars tested. They suggested that a liquid quick dip in 0.3% to 0.8% IBA would enhance the rooting response in a range of blackberry cultivars.

The objective of this study was to determine the effects of auxin treatment and cutting location along canes of three Arkansas thornless blackberry cultivars. The findings suggest that propagation of thornless erect blackberries by hardwood cuttings is feasible, and may have some utility. However, the results indicate that there are differing responses among cultivars with IBA rooting hormone. The findings indicated that although the quality of root systems may be enhanced with the use of rooting hormone, the percentage of rooted plants might be cultivar dependent. With this variation, one could speculate that different concentrations of rooting hormone may have variable effects on the rooting success of thornless erect blackberries. More research is necessary to achieve guidelines for propagating blackberries by hardwood cuttings.

Further studies of related interest may include research on plant establishment with regard to plant precocity and growth habits of blackberries propagated by hardwood cuttings. Blackberries are traditionally perennial plants that produce biennial canes. The first-year primocanes are vegetative, whereas the second-year floricanes are reproductive. We observed an interesting phenomenon of the blackberries growth habit that deviates from the traditional perennial plant/biennial cane system. We observed a morphological reversion of the floricane cuttings to juvenile plants after the plants rooted and flowered. As anticipated, the majority of cuttings that rooted subsequently produced flowers. However, after flowering was completed the canes did not die as expected. The plants continued to live and produce new vigorous shoots. We observed lateral shoots developing from vegetative buds along canes, as well as new shoots originating from the base of the plants. In concept, propagating by hardwood cuttings has enabled a blackberry plant to complete efflorescence, and then revert to the vegetative growth period. These observations suggest that propagating blackberries from hardwood cuttings may be of significant value and that more research of blackberry hardwood propagation is warranted.

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Table 1. Interaction means for cutting diameter (mm) of blackberry cultivar and cutting location along canes.

Cultivar	Apical	Mid	Basal	Main effect of cv.
Apache	4.2aC[zy]	4.7bB	5.5cA	4.8b
Arapaho	4.2aC	5.5aB	6.3aA	5.3a
Navaho	4.1aC	4.9bB	5.8bA	4.9b
Main effect of location	4.2C	5.1B	5.9A	-

[z] Means in columns followed by different lowercase letters are significantly different (P< 0.05).
[y] Means in rows followed by different uppercase letters are significantly different (P< 0.05).

Table 2. Interaction means of blackberry cultivar by auxin treatment for percent rooting and root rating (1 to 5 rating with 5 = extensive root system) after 5 weeks.

	Percent rooted			Root rating		
Cultivar	- Auxin	+ Auxin	Main effect . of cv.	- Auxin	+ Auxin	Main effect of cv.
Apache	38.1aA[zy]	47.6aA	42.8A	2.1bB	3.5aA	2.8a
Arapaho	47.5aA	14.2bB	30.9A	2.1bB	2.1bB	2.1b
Navaho	38.1aA	26.9bA	32.5A	2.6bB	4.2aA	3.4a
Main effect of auxin	41.3A	29.5B	-	2.3B	3.3A	-

[z] Means in columns followed by different lowercase letters are significantly different (P< 0.05).
[y] Means in rows followed by different uppercase letters are significantly different (P< 0.05).

Physicochemical properties and leaching behavior of eight U.S. long-grain rice cultivars as related to rice texture

Devon K. Cameron[*] *and Ya-Jane Wang*[†]

ABSTRACT

There are many long-grain rice cultivars produced commercially in the U.S.; however, little work has been done on correlating the structure and physicochemical properties of starch with their texture. The physicochemical properties, leaching behavior, and texture attributes of eight long-grain rice cultivars were studied. Differences were observed in the approximate composition of kernels, including crude protein (6.6-9.3%), crude lipid (0.18-0.51%), and apparent amylose content (25.5-30.9%). These cultivars also differed slightly in thermal properties, such as onset temperature (73.7° to 77.4°C) and peak temperature (78.8° to 81.9°C). Although they showed a similar pasting temperature, their peak viscosities ranged from 680 to 982 Brabender units. The amount and the molecular size distribution of the leached starch molecules varied greatly among the samples. The leached amylose, instead of the apparent amylose, was suggested to play an important role in cooked rice texture.

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MEET THE STUDENT-AUTHOR

Devon K. Cameron

I am a native of Springdale, Ark., and a May 2003 graduate of the Dale Bumpers College of Agricultural, Food and Life Sciences where I majored in food science. I received a scholarship to attend the University of Arkansas and have also received scholarships from Ozark Food Processor's Association and Gerber Foods. I was involved in the Food Science Club, Student Sierra Coalition, Odyssey of the Mind, Gamma Beta Phi honor society, and Alpha Zeta Fraternity.

I worked as a lab technician for Dr. Ya-Jane Wang, my faculty sponsor. She encouraged me to do this project for the research and writing experience. I submitted the paper to the Institute of Food Technologists Student Association's undergraduate research competition. I was accepted as one of six finalists to present the paper in Chicago. I now attend Graduate School at the University of Arkansas. I have learned many things and I am grateful for this valuable and rewarding experience.

INTRODUCTION

Unlike other cereals, rice is principally consumed as a whole grain. Therefore, the texture of the whole grain is of great importance to consumers. Rice is classified in the U.S. as long-, medium-, and short-grain based on kernel length and shape (Adair, 1980). Long-grain rice represents the majority of rice produced in Arkansas as well as in the U.S. Cultivar selection is generally based on grain and milling yields, lodging, maturity, disease susceptibility, and plant heights (Slaton et al., 1999). Diversification of rice cultivars is necessary because of their variation in adaptation to various locations.

Although commercial long-grain cultivars are high yielding and possess typical long-grain characteristics, the properties of cooked rice become less predictable because of diversification of cultivars. Studies have shown that the classical relationships between amylose content and the final properties of the cooked rice are not always consistent and the fine structure of amylose and amylopectin may be more responsible for the cooked rice texture (Ong and Blanshard, 1995; Perez and Juliano, 1979: Reddy et al., 1993). Water-unextractable amylose, instead of the total amylose content of rice, has been found to correlate with the pasting behavior and texture attributes of rice (Battacharya et al., 1978) and can be used as an index of the amylopectin structure (Reddy et al., 1993).

There are many long-grain rice cultivars produced commercially in the U.S. The objective of this study was to examine the physicochemical properties of milled rice and the structures of leached/extractable starch components (i.e. amylose and amylopectin) of eight long-grain cultivars in relation to their texture attributes. Results from this study would help explain any observed differences in processing, cooking, and eating characteristics among these cultivars.

MATERIALS AND METHODS

Materials. Rough-rice samples of cultivars Ahrent, Cocodrie, Francis, Cypress, Drew, Wells, XL7, and XL8 were obtained from the 2002 crop of various locations. 'Ahrent', 'Cocodrie', and 'Francis' were obtained from the University of Arkansas Research and Extension Center, Stuttgart, Ark. 'Cypress', 'Drew', and 'Wells' were obtained from the University of Arkansas Northeast Research and Extension Center, Keiser, Ark. Cultivars XL7 and XL8 were obtained from RiceTec Inc., Alvin, Tex. 'Ahrent', 'Cocodrie', 'Francis', 'Cypress', 'Drew', and 'Wells' are inbred lines and XL7 and XL8 are hybrid lines. All samples were dried under gentle drying conditions to elimi-

nate drying effects. Samples were stored in self-sealing plastic bags under ambient conditions before analysis. Samples of 150 g of rough-rice were dehulled in a dehusker (THU-35, Satake Corporation, Hiroshima, Japan). The brown rice recovered was weighed and polished for 30 s in a friction mill (McGill Miller #2, Rapsco, Brookshire, Tex.). The resulting milled rice was weighed and separated into head rice and broken kernels on a double-tray shaker table (GrainMan Machinery, Miami, Fla.) with 4.67-mm indentation on both trays. Only head rice kernels were used in the study.

Chemical Composition of Rice Flour. Head rice was ground into flour with a cyclone sample mill (Udy Corp. Ft. Collins, Colo.) fitted with a 100-mesh sieve. Duplicate samples of 2 g were placed in aluminum moisture dishes and dried at 130˚C in a convection oven for 60 min according to Approved Method 44-15A (AACC, 2000). Apparent amylose content was determined by iodine colorimetry (Juliano et al. 1981). Crude protein was measured by micro-Kjeldahl according to Approved Method 46-13 (AACC, 2000). Crude lipid was measured according to Approved Method 30-20 (AACC, 2000) with the following modifications: rice flour (4-5 g) was extracted with 70 mL petroleum ether by boiling at 135˚C for 20 min and rinsing for 30 min using a Soxtec system (Avanti 2055, Foss North America, Eden Prairie, Minn.). The difference between the weight of the cup containing the extracted oil and the original weight of the cup was calculated to obtain the weight of the extracted crude lipid. The percentage of crude lipid was defined as the weight of extracted lipid divided by the weight of the original sample.

Gelatinization Characteristics. Gelatinization properties were assessed by a differential scanning calorimeter (DSC) (Pyris-1, Perkin-Elmer Co., Norwalk, Conn.). Starch (approximately 4.0 mg, dry basis) was weighed accurately into an aluminum DSC pan and then moistened with 8 µL of deionized water using a microsyringe. The pan was hermetically sealed and allowed to stand for at least 1 h prior to thermal analysis. Samples were heated from 25º to 120ºC at a rate of 10ºC/min. Enthalpy, onset, and peak temperatures were computed automatically. Triplicate measurements were performed for each sample.

Pasting and Gelling Properties of Rice Flour. The pasting characteristics were determined with a 10% (w/w) rice flour slurry using a Micro ViscoAmyloGraph (C.W. Brabender Instruments, Inc., South Hackensack, N.J.) equipped with a 700-mg cartridge and operated at a speed of 250 rpm. The starch slurry was heated from 50˚ to 95˚C at a rate of 3˚C/min, held at 95˚C for 10 min, and cooled down to 50˚C at a rate of 3˚C/min. The starch paste prepared with the Micro ViscoAmyloGraph was used for the gel property measurement. The starch paste was stored at 5˚C for 24 h and then measured with a TA-XT2i Texture Analyzer (Texture Technologies Corp., Scardale, N.Y.) using texture profile analysis (TPA). The paste was poured into three aluminum dishes (75 mm diameter x 20 mm height). The rims of the dishes were extended with aluminum foil to increase the height of the gel 1 cm above the rim (Takahashi et al., 1989). The gel was compressed at a speed of pre-test 2.0 mm/s, test 0.2 mm/s, and post-test 0.2 mm/s, to a distance of 5.0 mm with a cylindrical probe (2.54 mm diameter x 2.54 mm height) under the TPA test mode. The peak force of the first penetration was termed hardness and the negative peak height during retraction of the probe was termed stickiness. Triplicate measurements were performed on each sample.

Leached Carbohydrate Composition in Cooking Water. The sample preparation followed the method by Ong and Blanshard (1995) with modifications. Milled rice (10 g) was cooked with 20 g deionized water in a boiling water bath for 15 min and the solubles in the supernatant were characterized by high-performance size-exclusion chromatography (HPSEC) (Waters Corporate, Milford, Mass.) without dilution following the method of Kasemsuwan et al. (1995) with modifications (Wang and Wang, 2000). The solubles were autoclaved at 121°C for 30 min and then sonicated for 20 s for molecular size analysis of native samples. The solubles were diluted with three-fold deionized water and then treated with isoamylase (crystalline Pseudomonas isoamylase, Hayashibara Biochemical Laboratories Inc., Okayama, Japan) for molecular size analysis of debranched samples.

Textural Attribute of Cooked Rice. Rice was cooked and evaluated following the method of Sesmat and Meullenet (2001). Five kernels of cooked rice were used for the compression test to determine the hardness with a TA-XT2i Texture Analyzer.

Statistical Analysis. Experimental data were analyzed by using the general linear models procedure (1999 version; SAS Software Institute, Inc., Cary, N.C.), and least significance differences were computed at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical Composition, Physicochemical Properties, and Textural Attributes. The chemical composition, physicochemical properties, and textural attributes of the eight rice cultivars are summarized in Table 1. Differences were observed in the approximate composition of rice kernels, including crude protein (6.6-9.3%), crude lipid (0.18-0.51%), and apparent amylose content (25.5-30.9%). The hybrids XL7 and XL8 had different chemical compositions from the other inbred cultivars with a slightly higher amylose content and significantly lower crude protein and crude lipid contents.

The gelatinization temperatures of most rice cultivars were around 76 to 77°C for onset and 80 to 81°C for peak temperature. 'Drew' had a much lower onset temperature of 73.7°C and peak temperature of 78.8°C. A larger variation was observed in gelatinization enthalpy ranging from 7.4 to 11.5 Joule/g. Again 'Drew' also showed a lower gelatinization enthalpy. Cultivars XL7 and XL8 had similar gelatinization properties as the other cultivars. Their differences in gelatinization characteristics suggested potential variation during processing. Rice with a lower onset temperature and a lower enthalpy is easier to cook and requires less energy for processing.

When the pasting properties of the rice flours were measured by Micro ViscoAmyloGraph, the cultivars showed distinct pasting profiles. The range of the pasting temperatures were from 73.3 to 76.2°C, peak viscosity from 680 to 982 Brabender units (BU), breakdown from 316 to 604 BU, and setback viscosity 395 to 523 BU. 'Cocodrie' had the highest pasting temperature and setback viscosity but the lowest peak and breakdown viscosities. 'Francis' had the highest peak and breakdown viscosities. 'Drew' had the lowest pasting temperature, which reflected its lower gelatinization temperature. Although all samples are long-grain cultivars with a higher amylose content, their distinct pasting properties suggested their differences in the fine structures of amylose and amylopectin because the pasting properties were mainly controlled by the starch component in rice flour. These results supported the previous reports (Ong and Blanshard, 1995; Perez and Juliano, 1979; Reddy et al., 1993) that amylose content alone was not appropriate to predict the rice properties.

The gelling properties of flour pastes after storage at 5°C for 24 h showed distinct differences among the eight cultivars. Both hybrids XL7 and XL8 almost existed as a separated group from inbred cultivars, although differences also existed among the inbred cultivars. XL7 and XL8 had the highest gel hardness and stickiness values twice as high as those of 'Ahrent' and 'Wells'. The low crude lipid content in XL7 and XL8 might partly explain their high gel hardness because lipids would interfere with starch molecule reassociation, thus retarding the retrogradation process. The gels of 'Cypress' and 'Drew' were harder while 'Cocodrie' had the highest stickiness among the inbred cultivars.

When the textural attributes of cooked rice kernels were evaluated, 'Francis' had the highest hardness while XL7, XL8, and 'Wells' had the highest stickiness. The discrepancy in hardness between flour gels and cooked kernels of different cultivars might be attributed to their differences in shear force. When a flour paste was prepared using Micro ViscoAmyloGraph, a constant shear was applied and the shear caused starch granules fragmentation as evidenced from the presence of breakdown in viscosity. The fragmentation of starch granules enabled more starch molecules to become solubilized in water, which then interacted with each other to form gel network structure. Thereafter the extent of starch fragmentation and the structures of starch molecules might determine the gel hardness and stickiness. In contrast, there was no shear involved in cooking rice kernels and most starch granules were assumed to be intact after the cooking procedure with some solubilized molecules. The cooked rice hardness therefore would be possibly dominated by the extent of starch swelling, the leached starch molecules, and/or the unextractable amylose content.

The molecular size distribution of the leached molecules varied greatly among the eight cultivars and each cultivar showed a characteristic profile compared against a native rice starch (Fig. 1). The number of degrees of polymerization (DP) above each peak represents the molecular size calculated from the pullulan standards. It was apparent that large starch molecules with DP > 1,000 leached out and solubilized in the cooking water along with small molecular-sized saccharides, presumably naturally present in rice kernels, and both amylose and amylopectin molecules leached out from starch granules. The presence of a peak with a DP of 24 was intriguing but its origin was not clear although possibly from breakdown of amylopectin branch chains. Both XL7 and XL8 had significantly lower amounts of small molecular-sized saccharides with DP < 24 and a larger amount of large molecules whereas the other cultivars contained a much higher concentration of small saccharides. The larger amount of solubilized starch molecules in XL7 and XL8 suggested that either the molecular structures of starch molecules (amylose and/or amylopectin) in hybrids were different from those of the inbreds and could leach out easier, or the starch molecules were not as tightly organized within the starch granules as others. 'Cocodrie' had the largest amount of saccharides with DP < 24 whereas 'Wells' had the smallest amount of large molecular size molecules.

Because of overlapping of amylose and small amylopectin molecules (Fig. 1), the starch molecules in the supernatants of cooking water were debranched with isoamylase and their molecular size distributions are presented in Fig. 2. The first small peak was the amylose component and the second large peak was the amylopectin. Within the amylopectin fraction, the first peak with a peak maximal at DP 33 was the long-branch chains and the second peak with a peak maximal at DP 18 was the short-branch chains. XL8 had the largest amylose peak followed by XL7, and 'Wells' and 'Ahrent' had lower leached amylose contents. Both XL7 and XL8 also had a much larger amount of leached amylopectin,

which distinctly separated them from the other cultivars. Based on the profiles and the refractive index response, XL7 and XL8 leached out the most starch molecules, and 'Wells' the least.

In order to identify any significant correlation among various physical and chemical properties, a statistical analysis was conducted and the results are listed in Table 2. Both protein and lipid were found to have a negative impact on the hardness of the flour gel, and the cooked rice with lipids showed higher correlation coefficients. This negative correlation supported the much greater gel hardness of XL7 and 'XL8 'because of their lower lipid contents. The presence of lipid could interfere with the retrogradation of starch molecules, particularly amylose, therefore rice with a higher lipid content showed a weaker gel and softer texture. Although apparent amylose content is commonly used as an indicator to predict the textural properties of rice cultivars, this study did not show a strong relationship between the apparent amylose content and the cooked rice hardness and stickiness of these. Nevertheless, the apparent amylose content significantly affected the pasting properties of rice flours, including setback viscosity (r=0.587), gel hardness (r=0.696), and gel stickiness (r=-0.746). The onset gelatinization temperature was not influenced by the apparent amylose content but negatively correlated with the lipid content. The gel stickiness positively correlated with the peak viscosity (r=0.621) but was negatively correlated with setback viscosity (r=-0.701). The leached amylose showed a stronger correlation with the cooked rice texture than did the apparent amylose. The higher the amount of leached amylose, the harder the final cooked rice texture. Although the leached amylopectin also showed a positive correlation with the hardness of the cooked rice, it is believed the leached amylose dominated the cooked rice texture due to its linear structure and greater tendency to reassociate.

Although all of the cultivars used in this study were long-grain, they had significant differences in physicochemical properties and cooked rice texture, which consequently would have an impact on their processing and food applications. The apparent amylose content was not suitable to predict the cooked rice texture but could serve as a good indicator for gel hardness of rice flour paste.

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TABLE 1. Chemical composition and physicochemical properties of milled rice flour and cooked rice hardness from rice cultivars Ahrent, Cocodrie, Cypress, Drew, Francis, Wells, XL7, and XL8.[z]

	Ahrent	Cocodrie	Cypress	Drew	Francis	Wells	XL7	XL8
Chemical composition								
Moisture (%)	9.0d	11.0a	10.5b	10.5b	10.7a	10.3b	10.3b	9.8c
Crude protein (%, db)	9.3a	8.3b	7.2c	6.6d	7.2c	6.8d	7.0cd	6.8d
Crude fat (%, db)	0.48b	0.50ab	0.38d	0.51a	0.33e	0.46c	0.18g	0.24f
Apparent amylose (%, db)	25.5c	30.9a	28.4abc	29.7ab	27.8bc	26.8a	30.4a	30.1ab
Gelatinization								
Onset temperature (ºC)	76.6c	76.5c	75.6d	73.7e	77.1b	77.4a	76.5c	77.0a
Peak temperature (ºC)	81.3ab	80.6cd	80.3d	78.8e	81.0bc	81.9a	80.3d	81.2b
Enthalpy (J/g)	7.4d	11.5a	9.0c	7.9d	8.6c	10.2abc	10.3b	10.4b
Pasting properties								
Pasting temperature (ºC)	75.5b	76.2a	74.3d	73.3e	74.9c	75.3b	74.9c	76.1a
Peak viscosity (BU)	817c	680d	909b	784c	982a	933ab	818c	765c
Breakdown (BU)	429d	316f	534c	427d	604a	570b	438d	402e
Setback (BU)	397d	523a	429bcd	446bc	410cd	395d	466b	448b
Gelling properties								
Hardness (g-force)	9.53e	13.70c	15.43b	16.00b	12.77c	11.00d	20.63a	19.37a
Stickiness (g-force)	3.03c	5.30a	3.47bc	4.03b	3.63bc	2.97c	6.23a	5.80a
Cooked rice properties								
Hardness (g-force)	5480f	6395cde	5985ef	6121def	7486a	6741bcd	7449ab	6996abc
Stickiness (g-force)	320c	228c	314c	452b	438b	604a	603a	598a

[z] Mean values in rows followed by the same letter are not significantly different at P < 0.05.

TABLE 2. Correlation matrix for data on chemical composition, physicochemical properties, and cooked rice hardness.[z]

	Protein	Lipid	Apparent amylose	Onset temperature	Peak viscosity	Setback viscosity	Leached amylose	Gel stickiness	Gel hardness	Cooked rice hardness
Lipid	0.403*									
Apparent amylose	0.36	-0.367*								
Onset temperature	-0.225	-0.834**	0.293							
Peak viscosity	-0.327	-0.132	-0.494*	-0.105						
Setback viscosity	0.319	0.053	0.587**	0.129	-0.981**					
Leached amylose	-0.358	-0.810**	0.580**	0.796**	-0.353	0.376*				
Gel hardness	-0.573**	-0.742**	0.696**	0.560**	-0.294	0.353*	0.844**			
Gel stickiness	0.129	0.576**	-0.746**	-0.607**	0.621**	-0.701**	-0.750**	-0.757**		
Cooked rice hardness	-0.432*	-0.568**	0.293	0.495**	0.202	-0.124	0.383*	0.367*	-0.339	
Cooked rice stickiness	0.601**	0.505**	0.12	-0.593**	-0.225	0.262	-0.415*	-0.407*	0.168	-0.409*

[z] Correlation coefficients followed by * and ** are significant at P < 0.01 and 0.001, respectively.

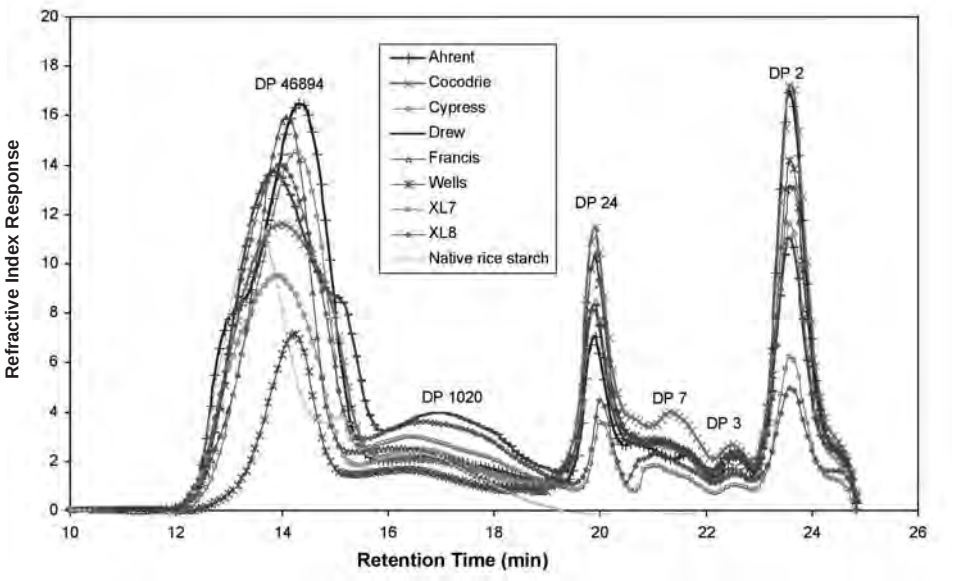

Fig. 1. Molecular size distribution of cooking water solubles analyzed by high-performance size exclusion chromatography. DP = degree of polymerization.

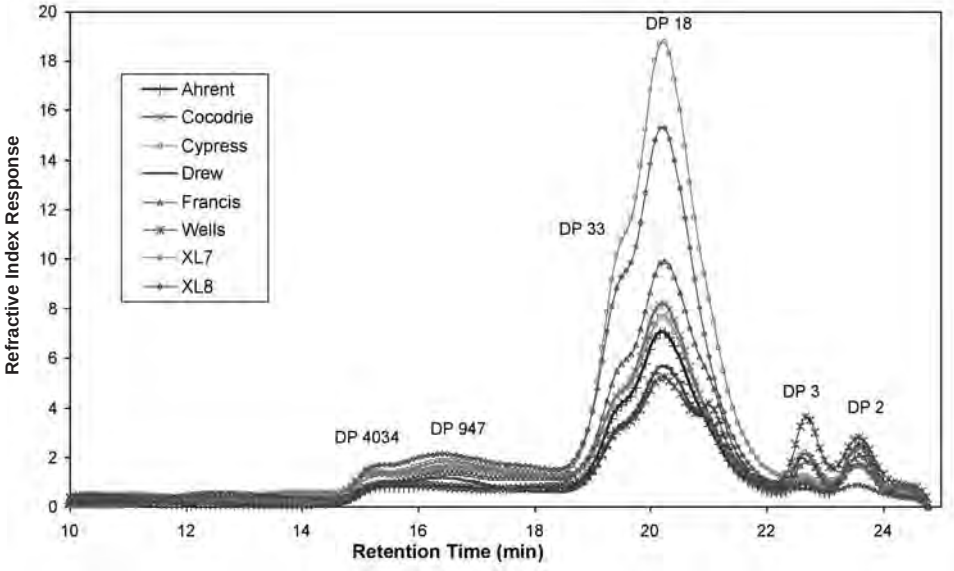

Fig. 2. Molecular size distribution of cooking water solubles debranched by isoamylase and analyzed by high-performance size exclusion chromatography. DP = degree of polymerization.

Watershed-scale agricultural land-use impact on instream physicochemical parameters

William H. Dillahunty[*], *Indrajeet Chaubey*[†], *and Marty D. Matlock*[§]

ABSTRACT

Nonpoint source (NPS) pollution is often the result of runoff losses from agricultural or urban areas. Even though the watershed approach to controlling NPS pollution is identified as the most efficient approach, data linking watershed scale land use and specific water quality implications are very limited. The objective of this study was to quantify the impact of agricultural land use on stream physico-chemical properties. The upper reach of Flint Creek was monitored at two sampling points draining an agricultural land. At each of these points, continuous measurement of stream characteristics such as temperature, dissolved oxygen (DO) concentration, depth, pH, and conductivity were taken at three different dates. Also, water samples were collected and analyzed for nitrogen (N) and phosphorus (P) concentrations to discern the impact of agricultural land use on water quality. The results indicated that nitrate N (NO_3-N) and phosphate P (PO_4-P) concentrations increased as the agricultural land use increased in the watershed. Fluctuation in the DO concentration also increased with higher agricultural land use. In order to help decrease the amount of nutrients introduced to the stream, a variety of best management practices (BMPs) could be implemented in the watershed.

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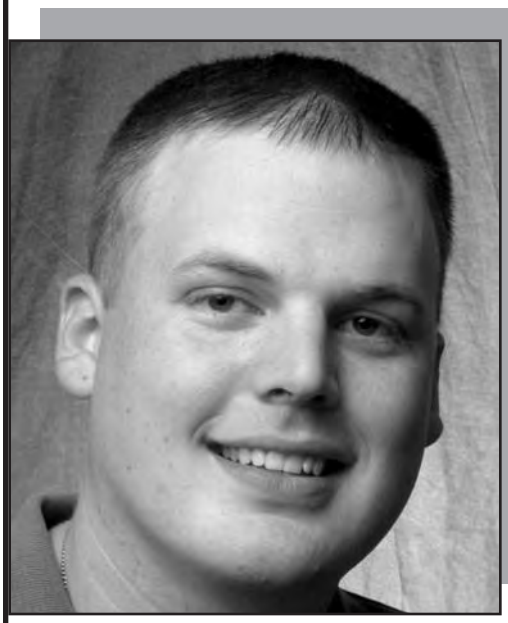
INTRODUCTION

Nonpoint source (NPS) pollution occurs when rainfall, snowmelt, or irrigation runs across land, picking up pollutants before entering a lake, stream, or river, carrying the pollutants to these water bodies (EPA, 1996). NPS pollution has emerged as the single largest source of pollution in the U.S., impairing 40% of assessed water bodies, 500,000 km of rivers and streams, and more than two million ha of lakes (Ritter and Shirmohammadi, 2001). A wide range of activities can result in NPS pollution, including: agriculture, forestry, septic systems, boating, construction, and urban runoff. Of these, however, agriculture is the primary source of impairment for rivers and lakes and third largest source of imapirment to estuaries. Agriculture is also identified as a major contributor to groundwater contamination and wetland degradation (EPA, 1996).

Some agricultural practices that can result in NPS pollution include: confined animal facilities, grazing, plowing, fertilization, planting, and harvesting (EPA, 1996). Sediment, nutrients, pathogens, and pesticides are principal pollutants resulting from these agricultural practices. Agricultural runoff can result in elevated nitrogen (N) and phosphorous (P) concentrations and can promote algal growth in lakes and streams, resulting in an increase in the microbial populations, and an increased oxygen demand by the photosynthetic organisms during the nighttime (Daniel et al., 1996). With this increased oxygen demand, there are lowered dissolved oxygen (DO) concentrations available to fish and other aquatic organisms. If these DO levels drop too low, fish kills can result, or at extremely low levels, anaerobic bacteria will begin the breakdown process, replacing the aerobic bacteria.

The EPA has identified the watershed approach as one of the most efficient ways to control NPS pollution. The watershed approach focuses within hydrologically defined geographic areas, taking into consideration both ground and surface water flow (EPA 1996). However, there is a need to understand the effect of land use on streamwater quality at a watershed scale before an effective NPS pollution control program can be designed. The objective of this research was to quantify linkages between agricultural land use, oxygen demand, and stream nutrient concentrations. This was accomplished by taking water quality measurements at two points along the same stream dominated by agricultural land use. By showing the linkages between agricultural land use and water quality, an effective watershed manage-

MEET THE STUDENT-AUTHOR



William H. Dillahunty

I graduated from Gentry High School in 1998. I then came to the University of Arkansas where I graduated in May 2003 with a B.S. degree in biological engineering as well as a minor in mathematics. I was raised on a dairy farm and still help out with all of the daily chores that keep it running. The stream that I chose for this research project passes through part of our farm.

I am a member of the American Society of Agricultural Engineers (ASAE). My team entered our senior design project in the AGCO National Student Design Competition in summer 2003 and was awarded first place for our Growth Chamber for Bio-Regenerative Life Support. This was a plant-growth chamber that researchers at NASA can use to simulate the atmospheric conditions on Mars. They can use it to find out how well plants will grow in a greenhouse there on future missions.

I am now pursuing a master's degree in biological engineering, during which I will be testing a furnace that can possibly be used to heat chicken houses. It will actually burn the chicken litter that has been removed from the houses for its fuel source. This may help with some of the problems that poultry farmers are facing.

ment plan to protect stream water quality can be developed.

MATERIALS AND METHODS

This study was conducted on the north fork of Flint Creek, located in Benton County, Ark. Arkansas Department of Environment Quality (ADEQ, 2002) has established water quality standards for streams within various ecoregions in Arkansas. This site lies within the Ozark Highlands Ecoregion (Table 1). All measured water-quality parameters at the two sites were within the acceptable levels set by the ADEQ.

Table 1. Acceptable levels for Ozark Highlands Ecoregion (ADEQ, 2002).

Parameter	Ecoregion Standard
Temperature	29°C
Dissolved O_2, <10 sq. mi.	Primary: 6 mg/L
	Critical: 2 mg/L
pH	6-9
NH_4-N	12.1 mg/L
NO_3-N	10 mg/L (drinking water)

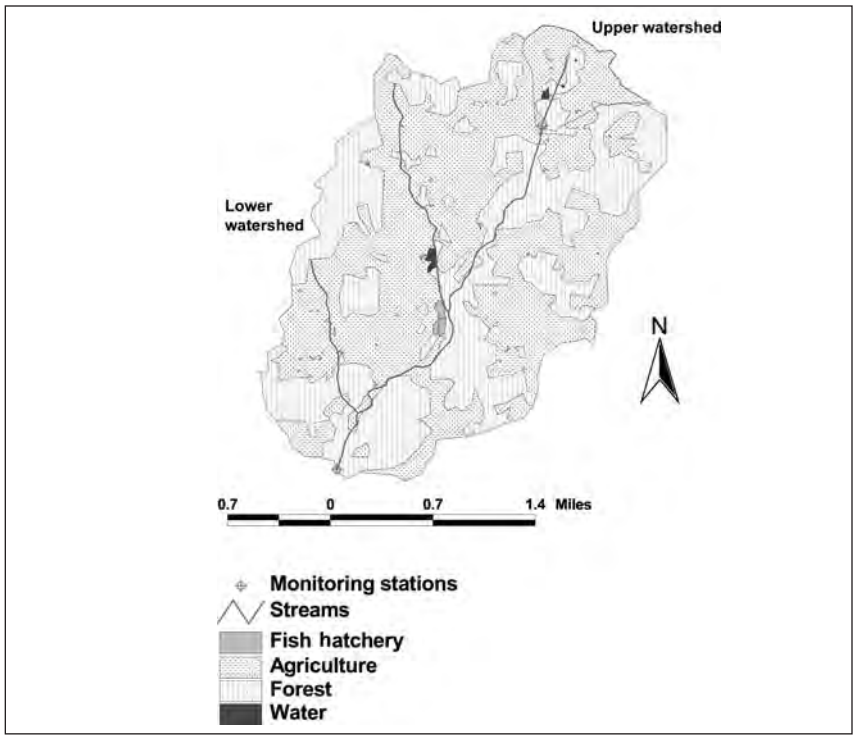


Fig. 1. Locations of sampling sites and land-use distribution within the watersheds.

The upstream sampling location was just below a pond at the source where the stream originates, while the second sampling site was located approximately 5.6 km downstream. The land use within each of the watersheds is predominantly agriculture.

The watersheds draining to the first and second sampling sites are named as the upper watershed and lower watershed, respectively. Location of the two watersheds, stream network, and locations of poultry and fish production facilities are shown in Fig. 1. Table 2 lists the watershed characteristics. The upper watershed contains 6.2% of the total watershed area.

ArcView GIS was used to delineate the watershed boundaries and to quantify watershed characteristics. GIS maps needed for the watershed included: the digital elevation map (DEM), land use map, stream network, road network, and the locations of fish and poultry production facilities. These maps were obtained from the Center for Advanced Spatial Technologies (CAST) at the University of Arkansas.

Water quality data were collected three different times during March and April 2003. Water quality sampling on 24 Mar. 2003 and 30 Mar. 2003 was under base flow condition and under storm flow condition on 19 Apr. 2003. YSI 600XLM data sondes were used to take measurements at 1 min. intervals for 24 h for dissolved oxygen, specific conductivity, pH, temperature, and water depth.

On each sampling date, two 20-mL water samples were collected at each site: one unfiltered, and one filtered using a 0.45 µm nylon-membrane filter. The filtering syringes were field-washed prior to sample collection. Immediately after collection, the samples were cooled, stored in the dark, and transported immediately to the laboratory for analysis of dissolved phosphorus (PO_4-P), nitrate nitrogen (NO_3-N), and ammonia nitrogen (NH_4-N).

Dissolved P was measured with an autoanalyzer using ascorbic-acid reduction, and total nitrate by cadmium-copper reduction method (APHA, 1999).

RESULTS AND DISCUSSION

Average physicochemical data collected at the two sites are shown in Table 3. Depth and flow rate increased significantly, while specific conductivity decreased at the lower watershed sampling site. The decrease in specific conductivity could be attributed to dilution and the increased flow. Average temperature and pH were similar at the two sampling sites.

Table 2. Land use within watersheds

Land Use:	Upper		Lower	
	Area (ha)	Fraction	Area (ha)	Fraction
Water	0.8	0.9%	2.8	0.2%
Forest	19.8	22.6%	586.1	41.6%
Field/Pasture	67.0	76.5%	821.1	58.2%
Total	87.6	100.0%	1410.0	100.0%

The temperature difference between the two sites was most likely caused by an increase in shading at the lower sample site due to a hillside near the stream, as well as increased tree canopy cover. Another factor that could be responsible for some of the difference in temperature is the stream depth. The stream flow depth was shallower at the upper site and had a lower flow rate, allowing a greater fluctuation in the diurnal stream temperature (Table 3). The temperature at the upper sampling site changed more rapidly than the lower site, throughout the day on 30 Mar. 2003 (Fig. 2). The specific conductivity of the water could be greatly affected by the differences in stream flow rates. With two other branches entering between the two sampling sites (Fig. 1), the water was diluted much more at the lower sampling site.

The increases in both nitrate and phosphate levels (Table 3) could be due to a variety of reasons but were most likely due to over-fertilization from the poultry houses within the watershed, or from stock cattle that were on many of the fields. Although an increase is noted in each of these levels, they are still well below the levels set by ADEQ to meet drinking water standards (Table 1).

Fig. 3 shows the dissolved oxygen (DO) concentrations over a 24-h period on 30 Mar. 2003. While the average DO concentrations were very similar for the two watersheds, Figure 3 shows the difference in diurnal cycle of DO, likely resulting from the presence of photosynthetic organisms. During the daytime, these organisms produce oxygen as a byproduct of photosynthesis, resulting in higher peak DO concentrations. During the nighttime hours, however, they consume oxygen, and cause greater instream DO depletion. The increase in nutrient concentrations present at the downstream site allows for greater algal growth and has a noticeable influence on the DO concentration cycle within the stream.

To help alleviate some of these problems, the use of commercial fertilizers may be more practical, where only the needed nutrients would be applied to the fields. With the majority of the pasture in this area being used for stock cattle, overgrazing may be another reason behind increased NPS pollution at the lower watershed. The careful use of commercial fertilizers and rotational grazing could help alleviate these problems. Other BMPs such as vegetative filter strips, removal of selected nutrients from the watershed, and chemical amendment of soils fertilized with animal manure could also be implemented to improve stream water quality.

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Table 3. Physicochemical water-quality data for the study sites.

			Averages (std. dev.)							
Sample	Date	Watershed	Depth (m)	pH	Temp (°C)	DO (mg/L)	Sp. cond. (µs/cm)	Ammonia (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)
1	3/24/03	Upper	0.14 (0.01)	7.5 (0.02)	15.8 (3.20)	9.3 (0.82)	1081 (3.8)	0.020	0.610	0.057
		Lower	0.31 (0.01)	7.6 (0.17)	15.2 (1.88)	9.0 (1.89)	224 (1.64)	0.036	3.390	0.090
2	3/30/03	Upper	0.28 (0.02)	7.6 (0.02)	9.8 (3.22)	10.6 (0.46)	1238 (14.6)	0.016	0.511	0.003
		Lower	0.47 (0.02)	7.7 (0.14)	9.5 (1.95)	10.9 (1.63)	223 (3.87)	0.012	2.865	0.013
3	4/19/03	Upper	0.10 (0.03)	6.9 (0.03)	15.5 (1.23)	6.1 (0.37)	306 (3.53)	0.050	0.420	0.001
		Lower	0.33 (0.03)	7.3 (0.11)	15.4 (0.78)	7.3 (1.41)	230 (3.71)	0.050	1.310	0.003

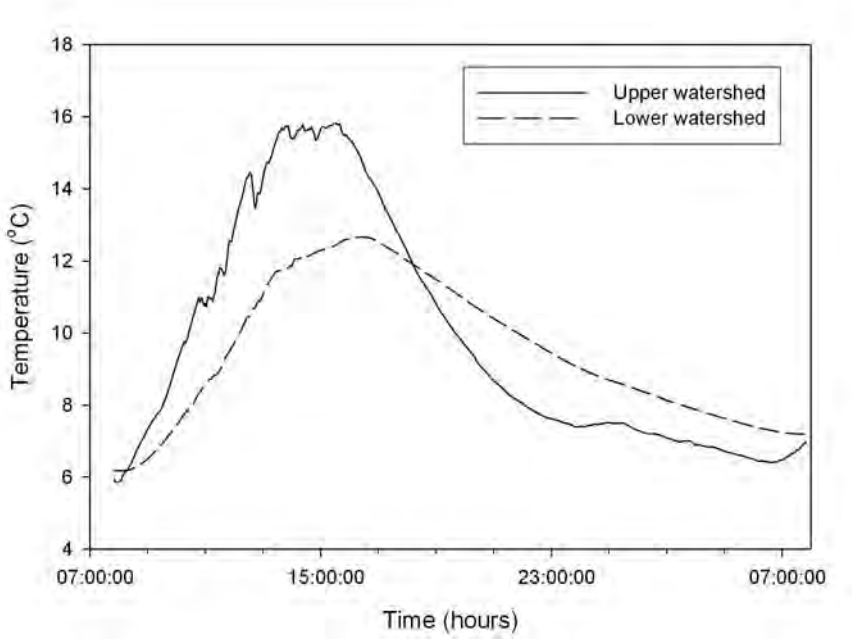

Fig. 2. Temperatures occurring during the 24-h period of sample 2.

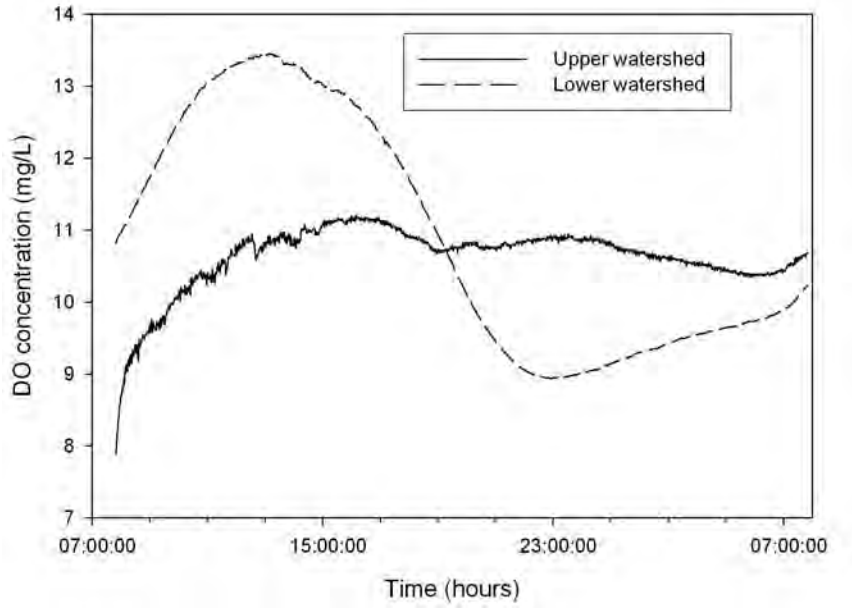

Fig. 3. Dissolved oxygen concentrations measured during the 24-h period of sample 2.

Chitosanase may enhance anti-fungal defense responses in transgenic tobacco

Bill L. Hendrix[*] *and James McD. Stewart*[†]

ABSTRACT

Chitosanase is an enzyme, similar to chitinase, capable of hydrolyzing the β-1,4-linkages between N-acetyl-D-glucosamine and D-glucosamine residues in partially acetylated chitosan polymers found in fungal cell walls. When attacked by pathogenic fungi, many plants exploit this hydrolytic action as a component of a larger post-attack defense response, but these enzymes may also play a role in the initial plant-pathogen interaction via the generation of elicitors resulting from the hydrolysis of fungal cell walls. To gain insight into these mechanisms, a *Paenbacillus* chitosanase was cloned, sequenced, and modified for plant expression. The modified gene was delivered to tobacco (*Nicotiana tabacum* L. cv. Xanthine) leaf disks via *Agrobacterium tumenfaciens*-mediated transformation. Whole plants were regenerated from the transformed cells. The putative transformants were tested for transgene integration, transcription, and translation. Confirmed transformants were then screened for enhanced responses to a *Rhizoctonia solani* cell-wall preparation by measuring time-course production of hydrogen peroxide, phenylalanine ammonia lyase, and peroxidase. These compounds play roles at different points in a pathogenesis-related signal transduction pathway and thus allow for an initial assessment of the global defense response. Preliminary data suggest that transgenic tobacco constitutively expressing a *Paenbacillus* chitosanase may activate pathogenesis-related defense responses more quickly than wild type tobacco.

[*] Bill L. Hendrix graduated in May 2003 with a B.S.A. degree in crop management.

[†] James McD. Stewart, faculty sponsor, is a professor in the Department of Crop, Soil and Environmental Sciences.

MEET THE STUDENT-AUTHOR

Bill L. Hendrix

I received a B.S.A. degree in crop management in May 2003. When I started college, a degree in agriculture wasn't one of my plans. In fact, I was a biology major for the first two years of my college career, but when I transferred to the University of Arkansas, I started working for a professor in the Crop, Soil, and Environmental Sciences Department, Dr. James McD. Stewart. I had never worked on a farm or even seen a soybean or cotton plant up close. Needless to say I was out of my element, but I worked hard and began to enjoy the work. It didn't take long for me to realize that agriculture is applied biology and many of the things I had already learned were directly applicable to the degree. I weighed my options and decided to switch degree plans. In hindsight, that decision was a good one. Many opportunities have come my way that I would have otherwise missed. This project is one such opportunity. Over its course, I was exposed to the world of research science and realized the agricultural industry is a career niche that suits me well.

I would like to thank Drs. Stewart, Srivastava, and de Los Reyes for fielding my numerous and sometimes bothersome questions, and my wife and daughter for helping me through my research frustrations and failures.

INTRODUCTION

Chitosanase (EC 3.2.1.132) is an enzyme widely distributed in nature. Its range of cellular functions centers primarily around its ability to hydrolyze β-1,4-linkages between N-acetyl-D-glucosamine (GlnAC) and D-glucosamine (Gln) residues in partially acetylated chitosan polymers found in fungal cell walls. The related enzyme chitinase has a similar fungal cell wall-degrading capability. As a result, many scientists have made efforts to exploit its action and increase plant fungal resistance by over-expressing this enzyme in various plant systems (Punja, 2001). To date, however, there has been only one report of plant transformation with a chitosanase gene (El Quakaoui et al., 1995) and no reports describing its in-planta anti-fungal potential.

No matter which cell wall-degrading enzyme is over-expressed, the degree of increased plant fungal resistance depends on two factors: 1) The efficacy with which the enzyme can degrade cell walls and, consequently, slow the attacking fungus; and 2) the number, length, and degree of acetylation of the oligomeric-carbohydrate elicitors released from the fungal cell walls. These factors are not mutually exclusive and may work together to achieve the realized plant resistance (Lorito and Scala, 1999).

Lorito et al. (1998) reported that for chitinases the source of the enzyme may influence its efficacy in degradation of GlnAC and Gln polymers. Over the past decade, the gamut of sources of cell wall-degrading enzymes has been tested. Most plant, bacterial, and fungal chitinases have offered plants varying degrees of protection from fungal pathogens, but generally, plant-derived chitinases have conferred inadequate control while bacterial and especially fungal chitinases have typically offered higher levels of resistance.

Glucosamine oligomers, released from fungal cell walls or crab shells after hydrolysis with a chitinase or a chitosanase (or by physical means) are known elicitors of plant defense responses such as stomatal closure (Lee et al., 1999); lignification (Vander et al., 1998; Moerschbacher et al., 1988); mitogen-activated protein kinase activation (Link et al., 2002); and pathogenesis-related (PR) gene expression (Jabs et al., 1997). The degree and type of responses elicited by these molecules depend on the length; degree of acetylation (DA) (Vander et al., 1998); and number of oligomers present. These factors may be as important as enzyme efficacy for increasing plant fungal resistance; however chitinase-cleaved oligomers may differ from those cleaved by a chitosanase. If acetylated vs. non-acetylated glucosamine residues were distributed randomly along the length of a

fungal cell-wall polymer, cleavage with chitosanase would produce oligomers that vary little from those produced by chitinase, but in some fungi the distribution is not random. Fukazimo et al. (1992) found the cell wall glucosamine polymer of *Fusarium oxysporum* f. sp. *lycopersici* had a DA of approximately 25-35%. In addition, they compared the degradation products produced by chitinase and chitosanase. The chitinase produced a GlnAC–Gln dimer as the primary product, whereas the chitosanase produced a relatively larger, heterogeneous pool of products that varied in length and DA. These results indicated that the acetylated glucosamine residues are clustered along the cell wall and that a chitosanase may be more efficient for cell wall digestion. Because only over-expression of chitinases has been examined in plant systems, in vivo variability in this area has not been tested.

In this study, a newly discovered *Paenbacillus* sp. 61427 chitosanase was expressed in tobacco (*Nicotiana tabacum* L. cv. Xanthine) to investigate the in-planta anti-fungal potential of this protein and to determine whether plant-signal transduction pathways can be enhanced by its elicitor-generating action.

MATERIALS AND METHODS

Bacterial Gene Isolation

A bacterium, identified by the sequence of the 16S rRNA gene, was discovered on a chitosanase screening plate (LB agar pH 7.9 + final concentration of 0.01% w/v chitosan dissolved in 0.1N HCl overnight). The bacterium produced a clear halo around the colony, indicating the presence of strong extra-cellular chitosanase activity. The bacterium was cultured and a genomic library was constructed with pGEM®-3Zf(+) (Promega, Madison, Wisc.) in *Escherichia coli* strain DH5α. The library was screened for chitosanase activity and one colony that exhibited strong activity was selected for further experimentation (Hendrix et al., 2001). The cloned plasmid, designated pCHN1, contained an insert of approximately 8 kb.

A nucleotide-deletion experiment (Promega, Madison, Wisc.: Erase-a base kit) was performed on the insert of pCHN1. The resultant clones began to lose chitosanase activity at time point 10, indicating nucleotides important for gene expression had been removed. The clones from time point 10 to16 were sequenced to identify the open reading frame and mature protein region of the chitosanase.

Gene Modification for Plant Expression

Specific primers were designed to 1) PCR-amplify the mature protein region of the chitosanase, 2) add an *Arabidopsis* extra-cellular chitinase signal peptide (Hasselhoff, 1992), and 3) generate BamHI (5') and SalI (3') restriction sites to facilitate further cloning.

The modified chitosanase fragment along with pHPT[1], a modified pUC19 vector containing a 35S Cauliflower Mosaic Virus (35S) promoter and nopaline synthase 3' transcription terminator (nos), were digested with BamH1/SalI and BamH1/AlwnI, SalI/AlwnI, respectively. The resultant fragments were used in a trimolecular ligation. The product, pERCSN, was cloned in *E. coli* strain DH5α for further manipulation.

pERCSN was digested with XbaI to liberate the experimental construct and ligated into the binary plant-transformation vector pPZP211 (Hajdukiewicz et al., 1994), also digested with XbaI and cloned in *E. coli*. The resultant *E. coli* colonies containing the experimental vector, pPZP-ERCSN, were screened by PCR and restriction digest to confirm proper gene size and orientation.

Tobacco Transformation

pPZP-ERCSN and an empty vector control were transferred to *Agrobacterium tumenfaciens* strain GV3101 by electroporation. Sterile tobacco (*N. tabacum* cv Xanthine) leaf disks were co-cultivated with *A. tumenfaciens* for 15 min in a Murashige and Skoog (MS) salt (4.314g/L)/ 3% sucrose (pH 5.9 with 1N KOH) solution for infection. Disks were then incubated for 3 d in the dark on MS/sucrose (same as above + phytoagar) plates without antibiotics. Following the co-cultivation period, disks were washed in MS/sucrose solution containing 400 µg/ml Timetin for 3 h to kill residual A. tumenfaciens and placed on shoot-initiation medium (SIM) (MS salt 4.314g/L, 3% sucrose, 0.5mg/ml naphthaleneacetic acid, 1mg/ml 6-benzylaminopurine, 300 µg/ml kanamycin, 250 µg/ml Timetin, 0.7% phytoagar) for callus and plantlet formation. Once plantlets regenerated and were 1 to 2 cm tall, they were removed with forceps and placed in rooting medium (same as SIM medium without hormones) for root formation. After two to three weeks, rooted plants were transferred to soil and grown under typical greenhouse conditions.

Analysis of the Integration Locus

Transgene integration was confirmed by Southern blot and hybridization. Total DNA was isolated from the regenerated plantlets following a modified procedure reported by Zhang et al. (2000). Ten micrograms of total DNA/plant were digested with EcoRI (Fig. 1) and size-separated by electrophoresis in 1% TAE agarose gels. The digests were transferred to Hybond N+ nylon membrane (Amersham Life Science, Piscataway, N.J.) with alkali blotting. Pre-hybridization and hybridization were performed according to Ausebel et al. (1995) with a ^{32}P-dCTP random prime-labeled csn probe. The membranes were washed twice with 2x SSC/0.1% sodium

dodecyl sulfate (SDS) followed by 0.2x SSC/ 0.1% SDS at 65°C until membrane signal was low. An autoradiograph was prepared by overnight exposure of Hyperfilm (Amersham Life Science, Piscataway, N.J.) to the gel at –80°C.

Transcriptional Analysis

Chitosanase transcription was assayed by Northern blot. Total RNA was isolated from young leaves with Trizol reagent (Gibco BRL, Grand Island, N.Y.). Five micrograms of total RNA were size-separated by electrophoresis in a 1% formaldehyde-agarose gel, transferred to a Hybond N+ nylon membrane, and cross-linked with UV irradiation. Pre-hybridization and hybridization were performed at 42°C with NorthernMax hybridization buffer (Ambion, Austin, Tex.) and a ^{32}P-dCTP random prime-labeled csn probe. Washing was at 42°C, but otherwise, washes and autoradiography were performed as described above.

Recombinant Protein Assay

Protein accumulation and activity were assayed with a leaf-disk lysoplate assay using a modified protocol previously described by Grenier et al. (1990). Briefly, agar slabs were augmented with 0.05% chitosan (pH 5.0 with 1N NaOH) dissolved in 0.1N HCl and 1% Triton-X 100. Fresh leaf disks were placed on the slabs and incubated at 28°C for 1 to 3 h to allow for diffusion of the extra-cellular chitosanase into the medium. The slabs where then stained with calcoflour white to visualize zones of chitosan lysis under UV light.

In vitro Anti-fungal Assay

The anti-fungal efficacy of the native, unmodified, bacterially produced chitosanase was compared to the recombinant chitosanase to assess the effect of gene modification and plant expression on the anti-fungal activity of the protein. *Rhizoctonia solani* mycelial plugs were centered on potato dextrose agar plates and challenged by transgenic leaf disks or by chitosanase buffer (10 mM phosphate pH 6.0) containing 5 µg, 0.5 µg, 0.05 µg, and 0 µg of partially purified, native chitosanase. After overnight incubation, growth inhibition was visually assessed.

Elicitor Preparation

Elicitors were prepared from *R. solani* and *Verticillium dalihae*[2] cell walls via methods described by Ke et al. (1998). Fungi were propagated in potato-dextrose broth with shaking at 27°C for 3 d. Mycelia were pelleted by centrifugation, washed with distilled water, and suspended in 1N NaOH (1:40 w/v mycelia to solution ratio). The suspension was then autoclaved for 15 min at 121°C to lyse cells and remove proteinaceous fractions. After a brief cooling period, insoluble material was pelleted by centrifugation, and the supernatant fraction was discarded. The pellet was washed with distilled water to remove residual NaOH, then resuspended in 2% acetic acid (1:100 w/v ratio) and again autoclaved 15 min at 121°C. The slurry was filtered through two layers of miracloth to remove acid insoluble material and collected for precipitation. Cell wall preparations were precipitated with 10M NaOH by raising solution pH to approximately 10. Precipitate was collected by centrifugation, washed four times with distilled water to remove any water-soluble fractions, and air-dried overnight. Elicitors were then dissolved in distilled water adjusted to pH 4.0 with acetic acid and stored at room temperature.

In-planta Elicitor Assay

The *R. solani* cell-wall preparation was applied to the surface of transgenic and wild-type tobacco leaves. Crude enzyme extracts (Moerschbacher et al., 1988) were taken at 0, 2, and 24 h after elicitor application, and phenylalanine ammonia lyase (PAL) and peroxidase (POD) activities were measured as described by Vander et al. (1998). All protein concentrations were estimated spectrophotometrically by the method of Bradford (1976).

Systemic H_2O_2 Production

Leaves from wild-type and transgenic plants were excised and imbibed with a 1mg/ml 3,3-diaminobenzidine (DAB) solution for 8 h. Elicitor preparations were then applied in solution through the cut petioles, and systemic H_2O_2 production was visually assayed on bleached leaves (boiled in 95% ethanol 10 min) at 0, 2, and 20 h after application as described by Orozco-Cardenas and Ryan (1999).

RESULTS AND DISCUSSION

Gene Discovery and Modification for Plant Expression

The original chitosanase-producing bacterium was identified as *Paenbacillus* sp. 61427 based on rRNA gene sequence. This bacterium produces a 259 aa, 29 kDa extra-cellular chitosanase. At the amino acid level, the *Paenbacillus* sp. 61427 chitosanase is 64% and 66% identical to *Bacillus circulans* and *B. ehimensis* chitosanases, respectively.

The *Paenbacillus* chitosanase mature protein gene was cloned and sequenced, and an *Arabidopsis* signal peptide, 35S Cauliflower Mosaic Virus promoter, and nopaline synthase terminator were added to obtain a 1676 bp experimental gene construct (Fig. 1) that was fused to the binary vector pPZP211 to obtain a 4409 bp T-DNA insert (Fig. 2).

[2] Fungi were courtesy of Dr. Craig Rothrock, University of Arkansas.

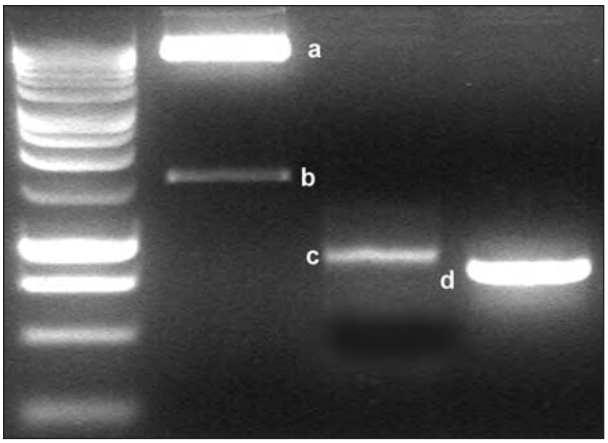

Fig. 1. 1% TAE agarose gel (EtBr stained) for confirmation of experimental vector. Ln1: 1kb ladder. Ln2 pPZPER-CSN HindIII digest; a-pPZP211 backbone 9014bp; b- experimental insert 1676bp. Ln3: PCR product, *Paenbacillus* chitosanase mature protein region + *Arabidopsis* signal peptide (ER sp); c- ER sp + csn 860bp. Ln4: PCR product, *Paenbacillus* chitosanase mature protein region; d- csn 777bp.

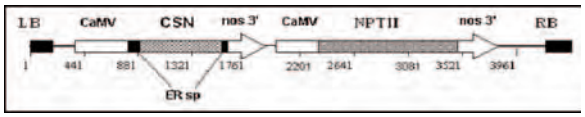

Fig. 2. 4409bp experimental T-DNA insert. Abbreviations: CaMV - 35S Cauliflower Mosaic Virus promoter; CSN - *Paenbacillus* chitosanase mature protein gene; ER sp - *Arabidopsis* signal peptide sequence; nos 3' - nopaline synthase 3' terminator sequence; EcoRI – recognition site; NPTII – neomycin phosphotransferase II gene; LB/RB – T-DNA left/right border sequences.

Transformation and Expression Confirmation

Numerous tobacco plantlets were regenerated, but gene transfer was tested in only 10 lines. Eight lines were confirmed transformed with varying copy number by Southern hybridization (Fig. 3A), and seven of the lines had high levels of csn mRNA as evidenced by Northern blot (Fig. 3B). Lines csn4, 5, 9, 10, and 11 were confirmed transformed by both Northern and Southern blots and were used for further experimentation or seed production. In addition, line csn3 was allowed to set seed to explore the possibility of gene reactivation in the segregating progeny of this multi-copy-silenced transgenic line. Before any line was used in an experiment, accumulation of active extra-cellular chitosanase was tested with a leaf-disk lysoplate assay (Fig. 3C).

Growth Inhibition of R. solani

Many times, bacterial proteins expressed in plant systems are heavily glycosylated or may be otherwise rendered inactive or less efficacious. To assess the effect of the gene modifications and plant expression on our bacterial protein, the fungal pathogen *R. solani* was challenged by the native and recombinant chitosanase. The partially purified native chitosanase dilutions inhibited growth at each concentration tested (Fig. 4A), and the transgenic leaf-disks from line csn5 inhibited growth at a level intermediate to the 0.5 to 0.05 μg level of the native chitosanase (Fig. 4B). No inhibition was observed with chitosanase buffer or wild-type leaf disks. These results indicated the recombinant chitosanase is expressed in our transgenic tobacco with in-planta activity levels sufficient for further experimentation.

In-planta Elicitor Assay

Lines csn5, csn6, and a non-transformed (NT) line (Fig. 3C) were assayed for a response to the *R. solani* cell-wall preparations. The cell walls were prepared to eliminate the small water-soluble oligomers known to elicit plant defense responses (Barber et al., 1994; Vander et al., 1998). As such, these polymeric glucosamine macromolecules required cleavage to produce soluble fragments capable of diffusing into plant tissues and triggering a plant defense response. The intent was to assay for enhanced plant perception of a simulated fungal attack in which wounding and extraneous proteinaceous elicitors were absent.

Line csn5 and line csn6 showed increases in both PAL and POD rates at 2 and 24 h. The PAL and POD rates for the NT line, however, remained unchanged over the course of the treatment (Fig. 5). POD activity in the transgenic lines was initially much higher than that of the NT line. We believe these results stem primarily from growing conditions. The transgenic lines were grown in small magenta boxes as a matter of convenience. The plants, though, had become quite large at the time of the experiments and were prone to moisture stress. While not visibly stressed at elicitor application, peroxidase levels may have been elevated due to an earlier moisture-stress response. The NT lines were grown under the same conditions but were much smaller and were never visibly moisture stressed. As such, they had normal POD activity at elicitor application. Despite minor discrepancies, these results suggest that constitutive extra-cellular expression of the *Paenbacillus* chitosanase may allow for enhanced plant perception of attacking fungi and thereby allow the plant to respond more quickly.

Systemic H_2O_2 Production

Lines csn5, csn6, and an NT line were evaluated for elicited H_2O_2 production in response to *R. solani* cell-wall preparations. Again, efforts were made to minimize wounding in order to assess only signal transduction events originating from elicitors released by the hydroly-

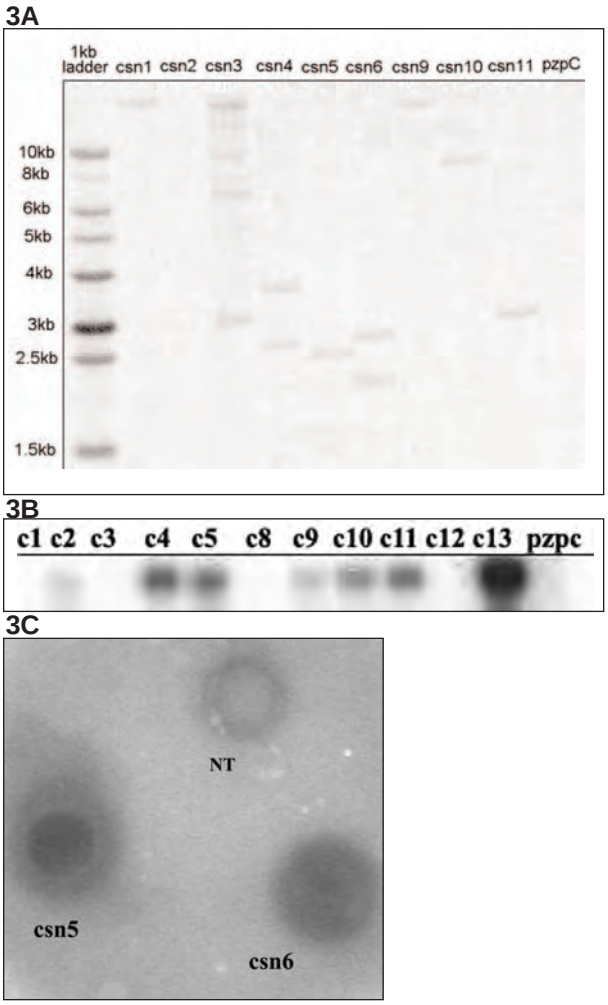

Fig. 3. Confirmation of transformation. A: Southern blot of ten transgenic lines and empty vector control (pzpC). One band = one copy of transgene B: Northern blot of 11 csn lines and empty vector control C: Example of leaf-disk lysoplate assay confirming accumulation of active, extracellular chitosanase in two transgenic lines.

sis of the applied fungal cell-wall preparation. Production of reactive oxygen species like H_2O_2 has been widely reported as one of the early events of plant defense response (Hancock et al. 2002; Vanacker et al., 2000). DAB forms a brown, insoluble polymer in the presence of H_2O_2 and peroxidase. As a semi-quantitative assay for H_2O_2, the leaves were allowed to take up a DAB solution through their petioles, exposed to the elicitor preparations, and visually assayed for the formation of the brown polymer and, hence, H_2O_2 production. Lines csn5 and csn6 both responded to the *R. solani* cell-wall preparation with systemic production of H_2O_2 at 2 and 20 h. The NT line, however, showed no response to the cell-wall preparation at 2 h and only a slight response at 20 h (Fig. 6). These results provide further evidence that

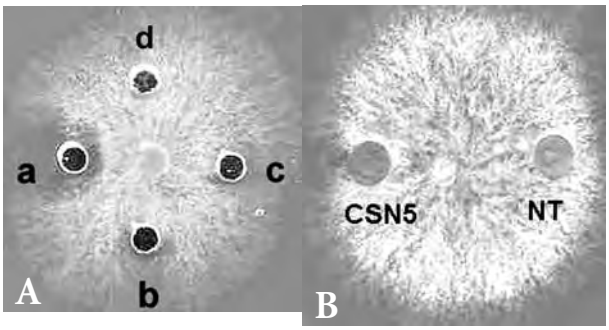

Fig. 4. Comparison of anti-fungal activity of the (A) native *Paenbacillus* chitosanase at 5 µg (a), 0.5 µg (b), 0.05 µg (c), and 0 µg (d) of total protein and (B) recombinant chitosanase from transformed leaf disks. The single copy line, CSN5, inhibited *R. solani* at levels near that of 0.05µg (c) of native protein.

transgenic tobacco constitutively expressing chitosanase may be capable of faster responses to attacking fungi than NT lines are.

In this study, the *Paenbacillus* chitosanase was transcribed, translated, and transported correctly in transgenic tobacco. Additionally, this enzyme enhanced the ability of transgenic tobacco to respond to fungal cell-wall-derived elicitors by cleaving these macromolecules into small fragments active as elicitors. This action may be able to increase plant fungal resistance by both lowering fungal infection efficacy and decreasing the time required for defense-gene induction.

ACKNOWLEDGMENTS

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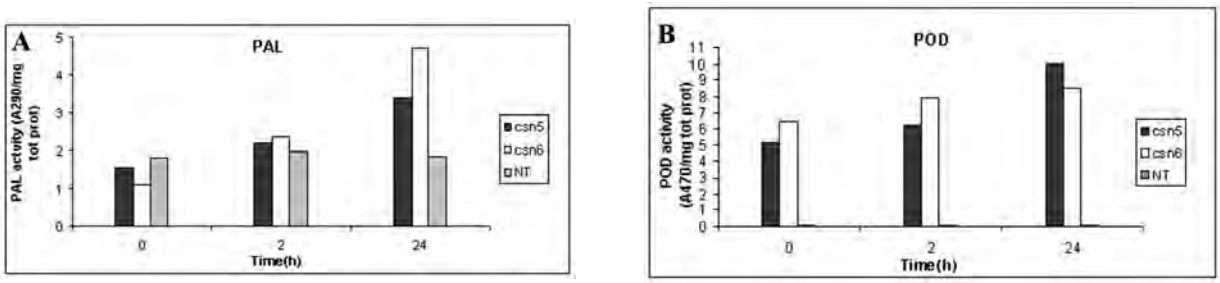

Fig. 5. Time-course changes in (A) PAL and (B) POD production in elicitor-treated tobacco leaves.

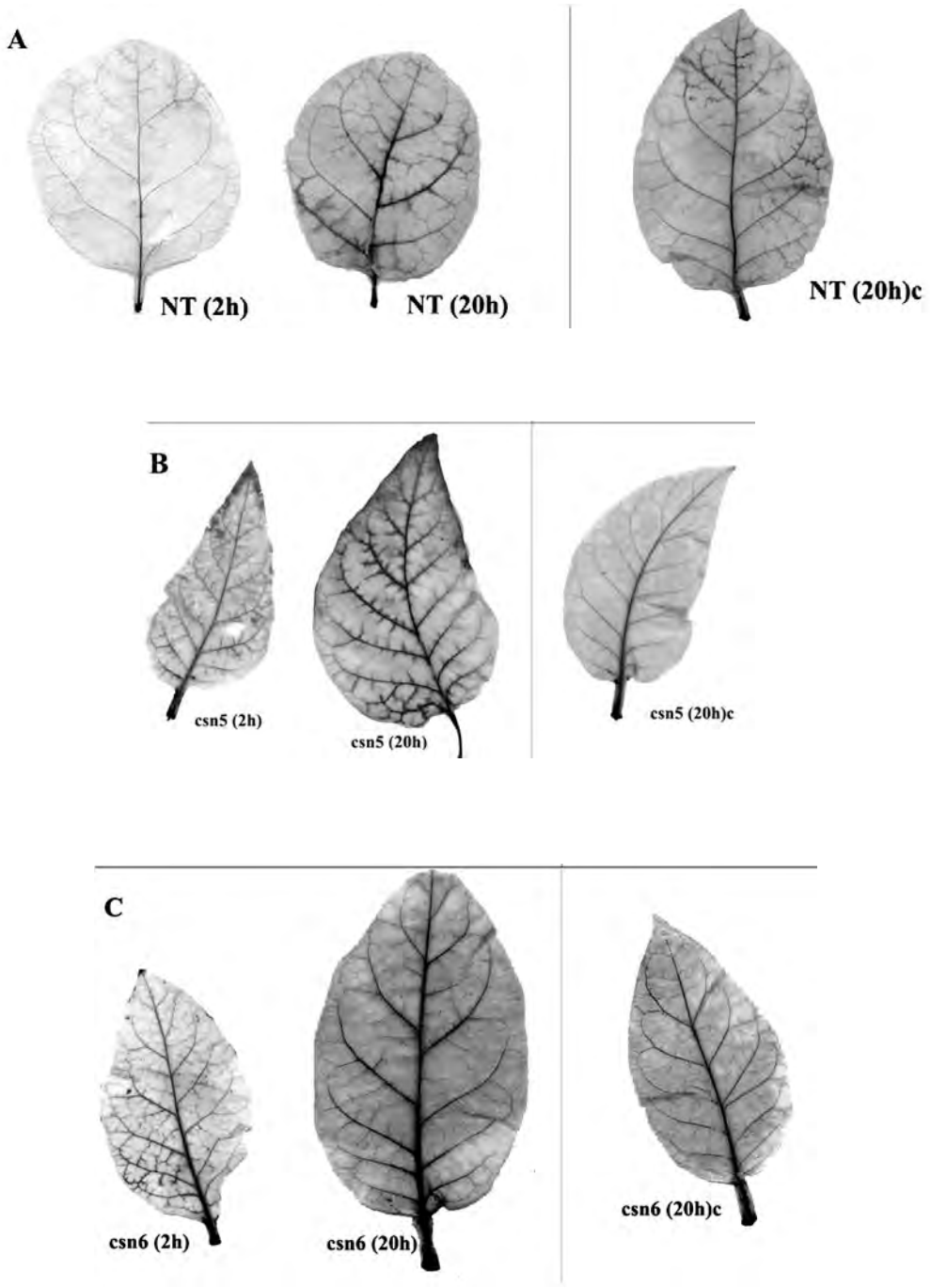

Fig. 6. Time-course changes in brown coloration due to DAB staining (H_2O_2 production) in the (A) NT, (B) csn5, and (C) csn6 lines at 2 and 20 h after treatment and at 20 h with DAB but no elicitor treatment [(20 h)c].

The effects of classic and variant infectious bursal disease viruses on lymphocyte populations in specific-pathogen-free White Leghorn chickens

Christina L. Johnson[*], *Ashley K. Cox*[†], *April D. Keeter*[§], *William J. Quinn*[∞], *Gisela F. Erf*[‡], *and Lisa A. Newberry*[‡‡]

ABSTRACT

Infectious bursal disease virus (IBDV) is a pathogen that primarily infects B lymphocytes in domestic avian species. This viral infection has been associated with immunosuppression, clinical disease/mortality, and enteric malabsorption effects. The purpose of this experiment was to compare the effects of a classic (USDA-STC) and a new variant IBDV (RB-4, known to induce primarily the enteric disease) on immune cell populations in lymphoid organs. Seventeen-day-old specific-pathogen-free (SPF) White Leghorn chickens were either not infected (control) or inoculated with either USDA-STC or RB-4 IBD viral isolate. On days 3 and 5 post-inoculation (PI), lymphoid tissues were collected to prepare cell suspensions for immunofluorescent staining and cell population analysis by flow cytometry. Portions of the tissues were snap frozen for immunohistochemistry to localize various immune cells and IBD virus in the tissues. Tissue homogenates were prepared to test for IBDV by quantitative MTT assay. Both the USDA-STC and RB-4 viruses greatly altered lymphocyte populations in the spleen and bursa. At 5 d PI, bursal B cells were approximately 25% and 60% of lymphocytes in chicks infected with USDA-STC and RB-4, respectively, whereas in control birds, B cells constituted 99% of bursal lymphocytes. This reduction in the proportions of bursal B cells was associated with an infiltration of T cells. In the spleen, IBDV infection also reduced the percentage of B cells and increased the percentage of T cells. The differential effects of classic and variant IBDV infection on immune cell populations in lymphoid organs may explain the differences in clinical effects induced by these viruses.

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[†] Ashley K. Cox is a sophomore in the Department of Poultry Science .

[§] April D. Keeter is a sophomore in the Department of Poultry Science.

[∞] Will J. Quinn was a senior in the Department of Biological Sciences.

[*†§∞] Students who conducted this interdisciplinary team research project during the Spring 2002 as part of the Rotations in Agricultural Laboratory Research course; Christina L. Johnson is the primary author.

[‡] Gisela F. Erf, teacher and faculty mentor regarding the immunology aspects of this project, is an associate professor in the Department of Poultry Science.

[‡‡] Lisa A. Newberry, teacher and faculty mentor regarding the virology aspects of this project, is an assistant professor in the Department of Poultry Science.

MEET THE STUDENT-AUTHORS

Christina L. Johnson, Primary Author

I am from Conway, Ark. I graduate from Conway High School in 2001 and I just completed my second year as a poultry science major. I heard about the Laboratory Rotations in Agricultural Research course during my first semester as a college student. I really liked the idea of learning about real research and actually conducting a research project, especially because I learn best by taking an active part in the learning process and I always liked the idea of working in a research laboratory. By taking the leadership role in preparing the manuscript for our team project, I learned about every aspect of research, including the background research, design of the project, actual hands-on experiments, team work, data analysis, and the many rewrites it takes to get a paper ready for submission. I learned a lot from this project and I am now conducting research in reproductive physiology with Dr. Keith Bramwell as part of my undergraduate program in poultry science. For the future I plan to go to graduate school.

Ashley K. Cox

I graduated in 2001 from Lincoln High School in Lincoln, Ark. A sophomore at the University in Arkansas majoring in poultry science with a minor in global agriculture, I plan to graduate with my B.S.A. degree in May 2005. During my time at the University of Arkansas I have been a part of many clubs including the Poultry Science Club and Sigma Alpha Professional Agriculture Sorority where I have served as the secretary for three semesters, and Arkansas Baptist Collegiate Ministry. During the spring or summer of 2004, I plan to study abroad in Scotland and further my knowledge of international agriculture. I have always been interested in science, and I believe that understanding and keeping abreast of current research is the key to being well informed in any area. With my experience in the research of the effects of the variant versus the classic IBDV, I gained some valuable skills, vocabulary, and insight that I will be able to refer back to as I further my education.

MEET THE STUDENT-AUTHORS, CONTINUED...

April D. Keeter

I am from Harrison, Ark. I graduated from Alpena High School in 2001 where I was valedictorian of my senior class. Throughout high school, I was an active member of Future Farmers of America and the BETA Honors Club. Based on my interest in animals and science, I enrolled in the Dale Bumpers College of Agricultural, Food and Life Sciences at the University of Arkansas. I recently completed my sophomore year in poultry science and pre-veterinary medicine. I enrolled in the Laboratory Rotations in Agricultural Research course during my freshman year because I wanted to know more about research and laboratory work. I liked this project because we could use the techniques we learned in the first part of the course and then apply them to a real disease problem in poultry. I know having this hands-on research experience will help me a lot with my science courses at the University of Arkansas and my studies in veterinary school.

I began my collegiate career at the University of Arkansas as a biology major without much of a focus on a specific niche to carve out for the future. This lack of specific focus continued until the final semester of my senior year. I always wanted to get involved in laboratory research but was not aware of the many opportunities available at the University of Arkansas. It was in my senior year that I, rather fortuitously, stumbled upon my first course in laboratory research and my first exposure to immunology research. I wished I had looked into opportunities to get involved in research earlier in my collegiate career. I learned a lot by conducting this multidisciplinary team project, including how much I love biomedical research, especially in immunology. I am now at the University of Pennsylvania pursuing a doctorate and further career in immunology.

William J. Quinn

INTRODUCTION

In chickens, as in mammalian species, the major players in protective immunity are T- and B-lymphocytes. In chickens, B cells develop in the bursa of Fabricius, which consists of numerous organized B-cell compartments (follicles) where pre-B cells differentiate into mature B cells. Mature B cells then leave the bursa to go to peripheral lymphoid organs such as the spleen where they can encounter foreign antigens and respond by producing antigen-specific antibodies. Antibodies to a particular antigen play a major role in the elimination of the antigen. T cells develop in the thymus gland where they differentiate into two major types of T-cell populations; helper T cells and cytotoxic T cells. Helper T cells, which can be identified based on their expression of CD4 molecules, play a major role in regulating the immune response providing activation factors to other T cells, B cells, and macrophages. Cytotoxic T cells, which can be identified by their expression of CD8 molecules, play an important role in killing virus-infected cells and tumor cells (cytotoxic T cells). Within these T cell populations there are further subsets that can be identified based on the type of antigen-receptor (T cell-receptor, TCR); the type of CD8 molecule ($\alpha\alpha$ or $\alpha\beta$); and the combination of CD4 and CD8 molecules.

Viruses that specifically infect T- or B- lymphocytes can have severe detrimental effects on the immune system and on the ability of an individual to defend against pathogenic microorganisms. The target cell for infectious bursal disease virus is the avian B cell. Infectious bursal disease virus (IBDV) is a double stranded RNA virus belonging to the family *Avibirnaviridae*. The virus particle is 60 nm in size and non-enveloped. There are three distinct age-associated disease syndromes that are linked to IBDV infection: immunosuppression, clinical/mortality, and malabsorption or enteric effects (Kim and Sharma, 2000). There are two recognized serotypes within this virus group as well as numerous variants of serotype I (Calnek et al., 1997). Within the classic

serotype I IBD virus group, clinical disease is associated with cytolysis, inflammation, edema, and heterophil infiltration, and viral infection is linked directly with immunosuppression (Allan et al., 1972). The variant serotype I IBD viruses induce similar but milder necrotic lesions, in the absence of acute inflammation. Newberry et al. (1997) isolated a variant IBDV (RB-4) from the proventriculus of a commercial broiler chicken; this variant induces mild tissue pathology, clinical signs, and associated enteric effects (Newberry et al., 1997). These differences in infection-associated symptoms may be due to the relative severity of B-cell loss and the type/effectiveness of the resulting IBDV-specific immune response.

At present, little is known with respect to how variant RB-4 IBDV differs from the classic USDA-STC IBDV during acute infection (e.g., one to 5 d PI). The objective of this study was to measure and compare the effects of the variant RB-4 (enteric origin) and classic USDA-STC IBD viruses on lymphocyte populations in spleen and bursa of specific-pathogen-free (SPF) White Leghorn chickens. Additionally, viral infection was confirmed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay and by localization of virus within affected tissues.

MATERIALS AND METHODS

Experimental Animals

In this experiment, 60 SPF White Leghorn chickens (Sunrise Farms, Catskill, N.Y.) were hatched at the University of Arkansas Poultry Health Laboratory. The chickens were randomly placed in five Horsfal isolation units with 15 chicks per isolation unit. Throughout the study, feed (prepared to meet or exceed National Research Council standards) and water were provided ad libitum. The environmental temperature was maintained at 35°C for the first week, followed by decreases of 2.8°C per week for the rest of the experiment. Group I was designated as the negative control, Groups II and IV were challenged with USDA-STC IBDV, and Groups III and V were challenged with variant RB-4 IBDV. The viral challenge inocula for both virus strains were standardized to a concentration of ~10^3 EID_{50}/mL and administered bilaterally to the eye (30 µL per eye). Chicks in Groups III and V, and chicks in Groups II and IV were inoculated with virus on days 17 and 19 post-hatch, respectively. On day 22 post-hatch (day 3 and 5 post-challenge), six chicks from each group (Group I-V) were randomly selected, weighed, humanely killed, and subjected to necropsy. Organ weights were recorded and tissue samples were collected for further analysis.

Immunofluorescent Staining of Spleen and Bursa Lymphocyte Suspensions and Cell Population Analysis by Flow Cytometry

For each chicken, individual single cell suspensions were prepared from spleen and bursa by gently pushing tissues through a 60 µm nylon mesh in ice-cold Dulbecco's phosphate buffered saline (PBS) with 10% calf serum. The bursal lymphocyte suspensions were placed in 15 mL Falcon tubes and centrifuged at 250 x g for 10 min. The supernatant fluid was removed, and the cell pellets were resuspended in PBS and washed again by centrifugation. After the final wash, pellets were resuspended in PBS+ (PBS with 1% bovine serum albumin and 0.1 % sodium azide) and the concentration was adjusted to 2 x 10^7 lymphocytes/mL. For spleen cell suspensions, lymphocytes were separated from red blood cells by slow-speed centrifugation following the procedure for whole blood described in Erf and Smyth (1996). Following isolation, splenic lymphocytes were then processed in the same manner as described for the bursal lymphocyte suspensions. To identify various types of lymphocyte populations, cells (1 x 10^6) were then stained with a panel of fluorescence- or biotin-labeled mouse monoclonal antibodies specific for chicken lymphocyte markers (Southern Biotechnology Associates, Inc., Birmingham, Ala.). Included were fluorescein isothiocyanate (FITC)-conjugated Bu-1 specific antibodies (Bu-1-FITC), CD4-FITC, CD8α-phycoerythrin, CD8β-biotin, TCR1-biotin, TCR2-biotin, and TCR3-biotin to identify B cells, CD4+ T cells (T helper cells), CD8+ T cells (cytotoxic T cells), T cells with γδ T cell receptors (TCRs), T cells with αβ1 TCRs, and T cells with αβ2 TCRs, respectively. The binding of biotinylated antibodies was detected using Quantum red-labeled streptavidin (Sigma Chemicals, St. Louis, Mo.). Additionally, to determine the proportions of lymphocytes in each cell suspension, lymphocytes were identified using unlabelled K55 mouse monoclonal antibody (a gift from Dr. H. S. Lillehoj, USDA-ARS, Beltsville, Md.) and goat-anti-mouse IgG-FITC antibody (Sigma Chemical Company, St. Louis, Mo.) in an indirect staining method. Immunofluorescent cell population analyses were then carried out using a FACSCalibur flow cytometer and the Cell Quest cell population analysis program (Becton Dickinson Flow Cytometry Systems, Mountain View, Calif.) as described by Erf et al. (1998). Data were expressed as the percentage of lymphocytes (K55+ cells) in each sample. The effect of treatment on the percentage of each cell population in a tissue was determined by analysis of variance (ANOVA). Differences between means were detected using Fisher's LSD test using the Systat 8.0® Statistical Analysis Software (SPSS Inc., Chicago, Ill.).

Viral Titration Assay

A viral titration assay was utilized to determine levels of viral infectivity in the tissues harvested at 3 and 5 d PI. A crude tissue homogenate was prepared from the spleen and bursa (1:5 w/v) in physiological saline (0.85%) containing antibiotics. Serial two-fold dilutions of each homogenate were then prepared in Eagle's Minimum Essential medium in sterile 96-well tissue-culture microtiter plates. Following sample dilution, each well of the microtiter plate was seeded with 100 µL of a BGM-70 cell suspension (1 x 10^6 cells/mL) (Jackwood et al., 1987). The plates were then incubated in a humidified incubator at 37°C in 5% CO_2 for 4 d. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma Chemical Company, St. Louis, Mo.) assay was performed based on the method of Mosmann (1983). Briefly, MTT was prepared at 5 µg/mL in 0.85% physiological saline, sterile-filtered, and dispensed 20 µL per well. Microtiter plates were then returned to the 37°C incubator for 2 h. Following incubation, BGM 70 cells were lysed using an acid/ethanol mixture and the MTT dye conversion levels (levels are directly related to relative amounts of living, metabolically active cells) were determined at an absorbency of 570 nm using a spectrophotometer (96-well Universal Microplate Reader, Bio-Tek Instruments, Winooski, Vt.).

Immunohistochemical Staining

To identify and localize virus and various lymphocyte populations in spleen and bursa, frozen tissue sections were prepared and stained as described by Erf et al. (1995). Briefly, the tissue samples of spleen and bursa were placed in labeled aluminum-foil cups with freezing medium (O.C.T. Compound, Tissue-Tek, Sacura Finetek, USA Inc., Torrance, Calif.) and snap frozen in liquid nitrogen. Tissues were stored at -80°C until use. For immunohistochemical staining, the frozen tissue samples were cut into 6 µm sections using a Microm Microtome Cryostat (MICROM Laborgeräte GmbH, Walldorf, Germany) and placed on poly-L lysine-coated microscope slides. To block non-specific binding sites on the tissues, the tissue sections were incubated over night in PBS 10% horse serum at room temperature in a humidified chamber. To identify and localize various lymphocyte populations in the tissues, sections were immunochemically stained using the same panel of lymphocyte-specific antibodies as described for immunofluorescent staining, except lymphocyte specific antibodies were not directly conjugated to a label. The presence and location of IBDV virus were detected using an IBDV-specific mouse monoclonal antibody (University of Maryland, College Park, Md.; Intervet, Inc., Millsboro, Dela.). The binding of unlabeled detection antibodies, all of which were mouse IgG antibodies, was revealed using horse-anti-mouse IgG antibodies conjugated with biotin. The binding of biotinylated antibodies was detected using peroxidase-labeled biotin-avidin reagent (ABC reagent, VECTASTAIN Elite ABC" staining kit, Vector Laboratories, Inc. Burlingame, Calif.). The binding of ABC reagent to the complex formed by detection antibody and biotinylated antibody was detected by adding DAB substrate, which was converted by the biotin-conjugated peroxidase enzyme into a brownish-red product that precipitated at the site formation. Tissue sections were then counterstained with Methyl Green stain, covered with glass cover-slips, and examined by bright field microscopy.

RESULTS AND DISCUSSION

This study was conducted to address how the immune system was affected by classic compared to variant IBDV during acute infection by focusing on the effects of IBDV infection on immune-cell population profiles in spleen and bursa.

Based on results obtained from quantitative MTT assay and immunohistochemistry (IHC), IBDV was present in experimental birds challenged with USDA-STC or RB-4 IBD viruses but not in uninfected controls (MTT data not shown, IHC see Fig. 1 d and h). Comparison of viral activity in tissue samples obtained at 3- versus 5-d PI, revealed higher and more consistent levels of viral activity at 5 d PI than at 3 d PI. Additionally, at 5 d PI, the level of viral infection in spleen and bursa was similar in chicks infected with classic and variant IBDV. Hence, results reported and discussed here will focus on results obtained at 5 d PI.

The body weights of all IBDV-challenged SPF chickens were lower than those of their age-matched control chickens (Table 1), which may be attributed to the infection- and/or immune response-associated loss in appetite, heightened metabolic activity, and potential enteric effects. Both absolute and body weight-adjusted spleen weights were higher in IBDV-infected chicks compared to non-infected controls. Spleen weights were not different in chicks infected with classic or variant IBDV (Table 1). This increase in spleen weight is likely due to the anti-IBDV immune responses initiated in the spleen. Antigen-specific immune responses in secondary lymphoid organs such as the spleen are generally associated with recruitment of lymphocytes from the circulation into the spleen and proliferation of antigen-specific lymphocytes. As shown by cell-population analysis, the proportions of T cells, particularly the proportions of the more sophisticated αβ T cells (compared to γδ T cells), increased in both groups of IBDV-infected chicks (Table 2). These T lymphocytes consisted of both helper T cells (CD4+CD8) and cytotoxic T cells (CD8αβ+). It

Table 1. Body weights (BW), lymphoid organ weights, and proventriculus weights in specific-pathogen-free White Leghorn chickens infected with classic (USDA-STC) or variant (RB4) infectious bursal disease virus (IBDV) at 17 d of age and examined 5 d post-infection.[z]

Variable	Control	USDA-STC IBDV	RB4 IBDV
Body weight (g)	130 ± 4.17 [a, y]	113 ± 4.77[b]	112 ± 4.52[b]
Bursa weight (g)	0.750 ± 0.053[a]	0.403 ± 0.026[b]	0.404 ± 0.035[b]
Bursa (%BW)	0.571 ± 0.028[a]	0.371 ± 0.036[a]	0.364 ± 0.032[b]
Spleen weight (g)	0.215 ± 0.018[b]	0.323 ± 0.023[a]	0.303 ±0.032[a]
Spleen (%BW)	0.165 ± 0.013[b]	0.289 ± 0.019[a]	0.267 ± 0.025[a]

[z] IBDV was administered by eye-drop, 30 µL of 10^3 EID_{50}/mL.

[y] Mean ± SEM based on six birds per treatment; for each weight, treatment means that do not share a common letter are different ($P \leq 0.05$).

is interesting to note that the viral infection-associated changes in the proportions among splenic lymphocytes differed between infection with USDA-STC and RB-4. The percentage of ab T cells in spleens from chicks infected with RB-4 was higher than in spleens from chicks infected with USDA-STC. Additionally, while infection with classic IBDV did not affect the proportions of CD4+CD8^{low} and CD8αα subpopulations, infection with variant IBDV resulted in increased proportions of these CD4- and/or CD8-defined lymphocyte subsets. Little is known about lymphocytes with either the CD4+CD8^{low} and CD8αα phenotypes in peripheral tissues such as the spleen. However, the recruitment of these unique cell types to the site of IBDV infection (IBDV localization data not shown) may in part be responsible for the less severe symptoms observed following infection with variant compared to classic IBDV. Similarly, the presence of these cell types may be an indication of a more effective immune response to IBDV infection. The notion that the presence of these lymphocyte subsets may be an indication of a more effective immune response is based on observations by Ward (2000), who reported the presence of these cell types in Rous sarcoma virus-induced tumors that were actively regressing.

Although the organization of the lymphoid areas in the spleen was affected by IBDV infection, including substantial reduction in the size of B-cell follicles and infiltration of T cells into B-cell areas (data not shown), the bursa was more severely affected by IBDV infection. IBDV infection with either the classic or variant strain decreased both the absolute and body weight-adjusted weight of bursae compared to non-infected controls. This effect on bursa weights was similar in both groups of IBDV-infected chicks and was associated with greatly reduced proportions of B cells (Table 3), less densely populated B-cell areas (follicles), and infiltration of T cells into follicles (Fig. 1 f and g). In uninfected controls, the bursa of Fabricius, which is the site of B-cell development, consists primarily of lymphocytes and more than 99% of these lymphocytes are B cells (Table 1). The few T cells isolated from bursae of uninfected chicks were primarily located in the spaces between the B-cell follicles and likely represent T cells present in bursal blood vessels (Fig. 1 a-c). Infection with either the classic or variant IBDV strain resulted in similar qualitative changes in bursal lymphocyte populations. However, the magnitude of these alterations differed between virus strains. Compared to infection with variant IBDV, infection with classic IBDV resulted in a greater loss of B cells.

Table 2. Proportions among lymphocyte populations in the spleen of specific-pathogen-free White Leghorn chickens infected with classic (USDA-STC) or variant (RB4) infectious bursal disease virus (IBDV) at 17 d of age and examined 5 d post-infection.[z]

Cell population (% total lymphocytes)	Control	USDA-STC IBDV	RB4 IBDV
B cells	40.2 ± 2.27[a, y]	10.6 ± 3.40[b]	9.82 ± 4.71[b]
γδ T cells	12.2 ± 0.93[b]	17.7 ± 1.46[a]	15.7 ± 1.51[ab]
αβ T cells	45.2 ± 1.62[c]	62.1 ± 2.02[b]	71.0 ± 6.06[a]
$CD4^+CD8^-$ cells	15.0 ± 1.09[b]	24.7 ± 3.00[a]	27.3 ± 1.85[a]
$CD4^+CD8^{low}$ cells	7.27 ± 1.45[b]	3.75 ± 1.84[b]	14.6 ± 5.27[a]
$CD8\alpha\beta^+$ cells	24.3 ± 2.48[b]	33.6 ± 4.00[a]	32.8 ± 2.41[a]
$CD8\alpha\alpha^+$ cells	13.7 ± 2.12[b]	10.4 ± 2.08[b]	21.2 ± 4.52[a]

[z] IBDV was administered by eye-drop, 30 µL of 10^3 EID_{50}/mL.

[y] Mean ± SEM based on six birds per treatment; for each cell population, treatment means that do not share a common letter are different ($P \leq 0.05$).

The loss of B cells due to IBDV infection was associated with extensive infiltration of T cells, particularly cytotoxic T cells. As viral presence was similar in bursae from both IBDV-infected groups, it is possible that heightened anti-viral cytotoxic activity mediated by infiltrating cytotoxic T cells may in part be responsible for the enhanced loss of B cells observed during infection with classic IBDV compared to variant IBDV. An important role of T cells in IBDV infection was recently demonstrated by Kim et al. (2000) and Rautenschlein et al. (2002). The exact contribution of T cells to the resolution and pathogenesis of IBDV infection, however, is not fully understood. Rautenschlein et al. (2002) support the role of the avian T cell as an IBD viral modulator by first limiting early-phase viral replication and secondarily increasing the level of tissue damage through the release of cytokines. Additionally, the study by Kim et al. (2000) supports a role of cytotoxic T cells in viral clearance.

In conclusion, both the classic USDA-STC and variant RB-4 IBDV strains had profound effects on the proportions among lymphocyte populations in the spleen and bursa at 5 d PI. The differences in the extent of alterations in immune-cell profiles of spleen and bursa observed with the classic versus the variant strain may be responsible for the differences in clinical symptoms induced by these viruses (i.e., immunosuppression and enteric effects versus primarily enteric effects, respectively). Further studies will be needed to characterize the role of the various T lymphocyte populations altered during acute IBDV infection.

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Table 3. Proportions among lymphocyte populations in the bursa of specific-pathogen-free White Leghorn chickens infected with classic (USDA-STC) or variant (RB4) infectious bursal disease virus (IBDV) at 17 d of age and examined 5 d post-infection.[z]

Cell population (% of total lymphocytes)	Control	USDA-STC IBDV	RB4 IBDV
B cells	99.9 ± 0.38[a, y]	23.9 ± 7.17[c]	58.1 ± 22.5[b]
γδ T cells	0.45 ± 0.05[b]	9.75 ± 1.06[a]	8.91 ± 2.52[a]
αβ T cells	0.56 ± 0.12[b]	54.1 ± 8.11[a]	40.9 ± 14.8[a]
$CD4^+CD8^-$ cells	0.33 ± 0.06[b]	18.5 ± 3.59[a]	16.5 ± 5.21[a]
$CD4^+CD8^{low}$ cells	0.11 ± 1.45[b]	2.74 ± 0.60[a]	4.23 ± 1.61[a]
$CD8\alpha\beta^+$ cells	0.21 ± 0.05[c]	25.3 ± 4.43[a]	13.1 ± 4.33[b]
$CD8\alpha\alpha^+$ cells	0.43 ± 0.07[b]	13.6 ± 1.12[a]	10.2 ± 3.69[a]

[z] IBDV was administered by eye-drop, 30 µL of 10^3 EID_{50}/mL.

[y] Mean ± SEM based on six birds per treatment; for each cell population, treatment means that do not share a common letter are different ($P \leq 0.05$).

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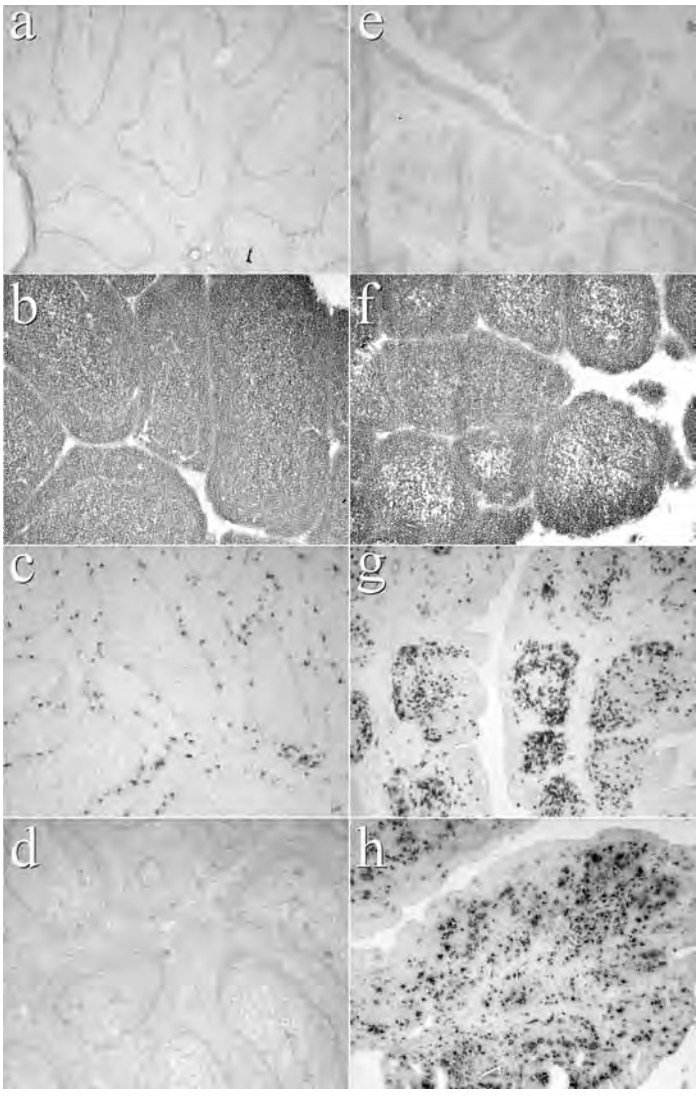

Fig. 1. Localization of B cells, T cells, and infectious bursal disease (IBD) virus in bursas from 22-day-old specific-pathogen-free White Leghorn chickens that were not infected or infected with variant RB-4 IBD virus 5 d prior to tissue collection. Immunochemically stained cells appear as dark cells on this black-and-white photograph. Pictures a-d and e-h represent bursal sections from uninfected and RB-4-infected chicks, respectively. Pictures (a) & (d) non-specific staining control (isotype control); (b) & (f) B cells; (c) & (g) T cells; (d) & (h) IBDV. The tissues were viewed by light bright-field microscope at 100x magnification.

Phase-feeding affects crude protein intake, excretion, and retention of broilers from 21 to 63 days

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ABSTRACT

An experiment was conducted to assess effects of phase-feeding (PF) on crude protein (CP) intake, excretion, and retention of broilers. Six birds were housed individually and were fed diets formulated using recommendations from National Research Council (NRC) or linear regression equations. Two PF treatments were prepared: standard (PF) and low (PF10), in which predicted lysine, sulfur amino acids, and threonine recommendations were reduced by 10%. For PF and PF10, two diets (high-nutrient and low-nutrient density) were blended in variable quantities to produce rations matching predicted amino acid requirements over two intervals. Birds were fed a single NRC grower and finisher diet or a series of PF and PF10 diets that were switched every other day. With the exception of weight gain, which was lower for PF than for birds fed the NRC and PF10 diets, no differences in growth performance were observed. Both PF diets reduced CP intake numerically from 21-43 d and 21-63 d, and significantly from 43-63 d ($P < 0.055$). Retention of CP was not impacted by diet although there was a tendency toward increased CP retention in birds fed the PF10 diet from 43-63 d ($P = 0.071$). Excretion of CP during the finisher period was reduced ($P < 0.05$) for birds fed PF and PF10 diets, and total CP excretion was numerically reduced (4.0% and 8.6%, respectively). These data indicate that in addition to economic benefits, PF may result in environmental benefits.

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INTRODUCTION

The National Research Council (NRC, 1994) provides amino acid (AA) recommendations for poultry producers, but these recommendations typically do not follow industry practices and are segregated into three periods (starter, grower, and finisher) that do not match those used in current production systems. Because broiler AA requirements decrease steadily with age, an opportunity exists to use phase-feeding (PF) as an alternative means of feeding broilers. Previous research (Pope and Emmert, 2002; Pope et al., 2002b; Warren and Emmert, 2000) indicated that PF did not negatively impact growth performance or carcass yield compared to broilers fed diets based on NRC (1994) requirements. Further, PF appeared to reduce dietary feed costs during the grower and finisher phases. By limiting excess dietary amino acids, PF may also reduce nitrogen excretion, as demonstrated previously with swine (Boisen et al., 1991). Reduction of nitrogen excretion is an environmental benefit and could potentially reduce ammonia production in broiler houses.

MATERIALS AND METHODS

All procedures were accepted by the University of Arkansas Institutional Animal Care and Use Committee. A trial was conducted to evaluate nitrogen excretion of broiler chickens fed: 1) a series of two diets formulated to contain NRC (1994) recommendations for lysine (Lys), sulfur amino acids (SAA), and threonine (Thr); 2) a series of 21 diets (PF) formulated to contain Lys, SAA, and Thr levels predicted by linear regression equations, with dietary AA levels lowered every other day; and 3) a series of 21 diets (PF10) formulated to contain 10% less Lys, SAA, and Thr than those contained in PF diets, with dietary AA levels lowered every other day. Prior to the experimental period (21 to 63 d), chicks were fed a common starter diet from 0 to 21 d that was formulated to meet essential nutrient recommendations (NRC, 1994). Regression equations from Emmert and Baker (1997) were modified to reflect male requirements (Emmert and Baker, 1997; Warren and Emmert, 2000) and were used to predict every other day PF (Treatment 2) as follows: digestible Lys, $y = 1.22 - 0.0095x$; digestible

MEET THE STUDENT-AUTHOR

I graduated in May 2002 with a B.S. degree in poultry science and currently am working on a master's degree in poultry science with an emphasis in nutrition. Dr. Jason Emmert was my undergraduate mentor for the crude-protein excretion study and is now my major professor for my graduate degree. I became involved with research while working in Dr. Emmert's lab. He was able to get me excited about conducting research experiments, and eventually I decided to get a master's degree under his supervision.

L. Niki Loupe

I graduated from Crossett High School in 1998 as an honor graduate. I was very active in high school clubs and organizations and immediately got involved in activities when I arrived at the University of Arkansas. As an undergraduate, I was fortunate to be one of our College Ambassadors and a Razorback Belle. I was also active in several organizations such as the Poultry Science Club, Golden Key National Honor Society, Associated Student Government, and Sigma Alpha Sorority.

As an undergraduate and graduate student, I have had the opportunity to compete nationally with my research at several scientific meetings. I recently received an award for my poster at the International Poultry Scientific Forum that was held in Atlanta, Georgia.

I am grateful to have had this opportunity to do research as an undergraduate. It helped me decide to get a master's degree in poultry nutrition after I graduated with a bachelor's degree.

methionine and cystine, y = (0.88 – 0.0063x)/2; and digestible Thr, y = 0.8 – 0.0053x, where y = digestible AA level, and x = midpoint (day) of the desired age range. Treatment 1 consisted of two NRC diets fed from 21 to 42 and 42 to 63 d (Table 1). In treatments two and three, an initial high-nutrient (HN) diet was formulated to contain predicted Lys, SAA, and Thr requirements for broilers from 21 to 23 d of age (Table 2). A low-nutrient (LN) diet was also prepared for treatments two and three, and was formulated to contain predicted Lys, SAA, and Thr requirements for broilers from 61 to 63 d of age (Table 2). Nineteen intermittent diets for treatments two and three were prepared by blending the respective HN and LN diets in variable quantities.

At 21 d of age, six male chicks were weighed, individually housed in wire cages, and assigned to each of the dietary treatments. Broilers were weighed at 63 d of age for determination of growth performance. Excreta were collected every other day to determine the impact of PF and PF10 on CP and AA excretion and retention. All excreta were collected in aluminum pans (placed under each cage), frozen, lyophilized, and analyzed for CP and AA.

This experiment was analyzed as a completely randomized design with individual birds considered the experimental unit. The General Linear Models (GLM) procedure of SAS® was used to conduct an analysis of variance on all data. When a significant main effect was detected, differences among treatment means were established using the least significant difference multiple-comparison procedure.

RESULTS AND DISCUSSION

The concept of PF is to frequently decrease dietary amino acid levels throughout grow-out; thus, PF may reduce CP excretion. Previous studies (Pope and Emmert, 2002; Pope et al., 2002a, b; Warren and Emmert, 2000) have shown no differences in growth performance between NRC (1994) and PF treatments. In addition, PF has shown the potential for economic benefits throughout the starter, grower, and finisher phases. In the current experiment, we assessed the impact of PF on CP intake, excretion, and retention.

Concurrent with this experiment, an experiment was conducted using the same diets with birds grown in floor-pens (Pope et al., 2002a). No differences ($P > 0.05$) in growth performance or carcass yield were observed among treatments, and performance was similar to the 18 birds used in the CP study. In the CP study, no significant differences ($P > 0.05$) in CP intake were observed during the grower or finisher periods (Table 3). However, overall CP intake was numerically reduced in birds fed PF and PF10 diets (5.2 and 6.3% reductions, respectively). Excretion of CP was not impacted by PF during the grower period, but PF and PF10 regimens led to significant ($P < 0.05$) reductions in CP excretion during the finisher period and numerical reductions overall (4.1 and 8.8% reductions, respectively; Table 3). Interestingly, with the exception of the grower period, CP retention was not impacted by diet.

Amino acid intake, excretion, and retention were analyzed for the overall experiment (21-63 d). No differences were seen for Lys intake or excretion among treatments, but Lys retention was significantly increased ($P = 0.05$) by PF and PF10 compared to the NRC treatment (Table 4). For Cys, intake and excretion were not affected by diet, but retention was numerically improved by PF and PF10 ($P = 0.07$). Threonine intake was reduced ($P = 0.04$) by PF and PF10, but no differences among treatments were observed for Thr excretion and retention.

Previous research has not been conducted with broilers to evaluate the impact of PF on crude protein intake, excretion, and retention. However, Boisen et al. (1991) reported decreased nitrogen excretion with pigs fed a nutritional regimen similar to our PF approach. Considerable research efforts are ongoing to reduce nutrient excretion and thereby lessen the impact of animal production on the environment. Although differences in CP excretion in this experiment may appear minimal on a per-bird basis, when applied on a commercial scale the potential impact could be tremendous. For instance, using our values (Table 3), total crude protein excretion for a broiler house containing 20,000 birds (grown to nine weeks of age) would be reduced by 560 and 1,200 kg by PF and PF10, respectively. Over the course of one year, PF and PF10 would be expected to reduce CP excretion (of birds in a single broiler house, assuming four growth cycles per year) by approximately 2,240 and 4,800 kg, respectively.

In summary, PF supports growth performance when compared to the NRC (1994) requirements and appears to decease CP excretion. Along with the potential economic benefits previously reported, decreasing CP in the excreta could have a tremendous environmental benefit. Phase-feeding is a program that should be considered by poultry producers as a way to decrease cost and lessen the impact of poultry production on the environment.

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Table 1. Composition of experimental diets fed to broilers from 21 to 63 d of age.

Ingredient	NRC[z]	NRC[z]	PF HN[y]	PF LN[y]	PF10 HN[x]	PF10 LN[x]
	(%)					
Corn	61.99	68.02	59.34	73.21	61.51	77.38
Soybean meal	31.69	25.67	37.32	20.57	32.17	16.37
Poultry fat	3.00	3.00	3.00	3.00	3.00	3.00
Vitamin mix[w]	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix[w]	0.10	0.10	0.10	0.10	0.10	0.10
Dicalcium PO_4	1.20	1.20	1.20	1.20	1.20	1.20
Limestone	1.30	1.30	1.30	1.30	1.30	1.30
NaCl	0.30	0.30	0.30	0.30	0.30	0.30
Choline Cl (60%)	0.10	0.10	0.10	0.10	0.10	0.10
L-Lysine•HCl	---	---	---	---	0.020	0.041
DL-Methionine	0.069	0.038	0.135	0.016	0.102	0.003
L-Threonine	0.047	0.072	0.003	0.003		0.009

[z] NRC diets contained Lys, SAA, and Thr levels recommended by NRC (1994).

[y] Phase-feeding (PF) diets were formulated to contain Lys, SAA, and Thr levels predicted by linear regression equations (Table 2) for 21-to-23-d-old [PF high-nutrient (HN)] or 61-to-63-d-old [PF low-nutrient (LN)] broilers. Experimental diets were produced by blending PF, HN, and PF LN diets in variable quantities (see Materials and Methods).

[x] Adjusted phase-feeding (PF10) diets were predicted by linear regression equations and lowered by 10% (Table 2) for 21-to-23-d-old (PF10 HN) or 61-to-63-d-old (PF10 LN) broilers. Experimental diets were produced by blending PF10 HN and PF10 LN diets in variable quantities (see Materials and Methods).

[w] Emmert et al. (1999).

Table 2. Calculated digestible amino acid levels fed to broilers from 21 to 63 d.

Diet	Days	Digestible content, % of diet[z]				CP,	ME_n,[y]
		Lysine	Methionine	Cystine	Threonine	%	kcal/kg
NRC[x]	21 to 42	0.88	0.33	0.31	0.66	19.6	3,114
NRC[x]	42 to 63	0.75	0.30	0.26	0.60	17.4	3,169
PF HN[w]	21 to 23	1.01	0.37	0.37	0.68	21.8	3,062
PF LN[v]	61 to 63	0.63	0.24	0.24	0.47	15.4	3,218
PF10 HN[w]	21 to 23	0.91	0.33	0.33	0.62	19.8	3,109
PF10 LN[v]	61 to 63	0.57	0.22	0.22	0.42	13.8	3,256

[z] Digestible amino acid, CP, and dietary ME content calculated from the analytical values for total Lys, SAA, and Thr in corn and soybean meal and published digestibility coefficients.

[y] Metabolizable energy values for corn, soybean meal, and poultry fat were assumed to be 3,350, 2,440, and 8,800 kcal MEn/kg, respectively.

[x] Although the NRC (1994) provides total dietary AA recommendations, digestible AA levels were calculated after formulation of diets to meet total NRC (1994) recommendations for dietary Lys, SAA, and Thr.

[w] Phase-feeding high-nutrient (PF HN and PF10 HN) diets were formulated to contain Lys, SAA, and Thr levels predicted by linear regression equations for 21-23-d-old broilers. Experimental diets were produced by blending HN and LN diets in variable quantities.

[v] Phase-feeding low-nutrient (PF LN and PF10 LN) diets were formulated to contain Lys, SAA, and Thr levels predicted by linear regression equations for 61 to 63-d-old broilers. Experimental diets were produced by blending HN and LN diets in variable quantities.

Table 3. Crude protein intake, excretion, and retention of broilers from 21 to 63 d.[z]

Treatment	Grower period (21 to 43 d)			Finisher period (43 to 63 d)			Overall (21 to 63 d)		
	Intake	Excretion	Retention	Intake	Excretion	Retention	Intake	Excretion	Retention
	(g)	(g)	(%)	(g)	(g)	(%)		(g)	(g) (%)
NRC	710	314	56	728	371	49	1439	685	53
PF	666	319	52	697	338	52	1364	657	52
PF10	676	317	54	672	308	54	1348	625	54
Pooled SD[y]	77	49	4.0	38	32	4.2	89	73	2.8
NRC vs PF and PF10			P = 0.06			P = 0.02			

[z] Values are means of six broilers housed individually.

[y] SD = Standard deviation.

Table 4. Amino acid intake, excretion, and retention of broilers from 21 to 63 d.[z]

Treatment	Lysine			Methionine			Cysteine			Threonine		
	Intake	Excretion	Retention	Intake	Excretion	Retention	Intake	Excretion	Retention	Intake	Excretion	Retention
	(g)	(g)	(%)	(g)	(g)	(%)	(g)	(g)	(%)	(g)	(g)	(%)
NRC	73.3	11.6	84.3	26.0	3.60	86.2	24.5	5.54	75.5	55.7	12.2	78.1
PF	74.3	10.3	86.1	26.3	3.54	86.5	24.4	5.52	77.4	51.0	12.0	76.5
PF10	72.7	9.90	86.5	26.3	3.58	86.5	25.7	5.58	78.5	51.9	11.4	78.1
Pooled SD[y]	4.72	1.80	1.74	1.72	0.71	2.20	1.58	0.83	2.28	3.35	1.72	2.21
NRC vs PF and PF10			P = 0.05						P = 0.07	P = 0.04		

[z] Values are means of six broilers housed individually.

[y] SD = Standard deviation.

Changing career paths in environmental, soil, and water science and crop management: Survey results

Thalia M. Madewell[*]*, Kristofor Brye*[†]*, and Mary C. Savin*[§]

ABSTRACT

Periodic assessment of goals is critical to maintaining a successful academic program. Two surveys were designed and distributed to alumni and potential employers of graduates of the Department of Crop, Soil, and Environmental Sciences (CSES) in the summer of 2002 to assess the profitability of advanced degrees in terms of earning potential and to determine sectors of the workforce currently employing department graduates. The CSES Alumni Satisfaction Survey was sent to 792 department alumni, including graduates of the previous Agronomy program as well as the current Crop, Soil, and Environmental Sciences program. The CSES Employer Survey was distributed to 281 professionals and provided valuable information concerning current workforce requirements of college graduates. Survey results suggested that alumni career paths are changing; alumni are employed quickly, but fewer alumni are self- or government-employed while more are working in industry positions. Results from the CSES Employer Survey suggest that alumni are earning salaries typical for this region. Alumni salaries also indicated that advanced degrees are profitable in that the number of alumni reporting higher-end salaries increased to a greater extent among M.S. and Ph.D. graduates. These results will be used to enhance recruitment of prospective students and to improve advisement of current departmental students. Ultimately, the CSES Department hopes to increase enrollment, student satisfaction with degree programs, and career preparedness.

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MEET THE STUDENT-AUTHOR

Thalia M. Madewell

I am a 1999 graduate of Muskogee High School in Muskogee, Okla. After visiting several universities in Oklahoma, I began my undergraduate education as an environmental, soil, and water science major at the University of Arkansas in the fall of 1999 and immediately knew that I had made the right choice. I was awarded several academic scholarships during my time at the University of Arkansas including the University Scholarship, Bobby R. Wells Memorial Scholarship, Adair Scholarship in Agronomy, Dale and Wilhelmina S. Hinkle Scholarship, and the Harold and Iva Hicks Scholarship. I was also awarded a Dale Bumpers Undergraduate Research Grant to conduct work on this project. In addition to academic interests, I have tried to remain active in extracurricular activities such as the Crop, Soil, and Environmental Sciences Undergraduate Club and I am also an alumnus of Kappa Delta Sorority.

I began work on this project in May 2002 and was afforded the opportunity to present my research to the Arkansas Environmental Federation, the Arkansas Academy of Science, and the Crop, Soil, and Environmental Sciences Departmental Seminar. Throughout my work, I have gained invaluable experience in conducting, preparing, and presenting research. I graduated in May 2003 and hope to begin work on a Master of Science degree in Crop, Soil, and Environmental Sciences in 2004.

INTRODUCTION

All academic programs need periodic reviews to evolve in response to advances in technology, changing societal needs, and overall increased states of knowledge. Assessment is especially needed in higher education to provide accountability for funds, to ensure a well-trained workforce, and to improve the effectiveness of academic programs (Miller et al., 1998). A comprehensive review including department graduates, as well as their current and potential employers, can prove a sound tool in judging overall program effectiveness. While employers can attest to the quality of graduates and, therefore, the quality of a program, alumni offer a unique perspective to judge the strengths and weaknesses of a program (Cole and Thompson, 2002; Miller et al., 1998). Alumni of agriculturally related programs are particularly important targets because the degree of satisfaction former students have with their education is an indication of the success of an agricultural program (Barkley, 1993). If students do not receive what they perceive to be a worthwhile investment, then agricultural programs will inevitably lose students to other more valuable programs.

Other academic institutions have recognized the need to maintain an on-going dialogue with potential employers to gauge the quality of graduates' knowledge, skills, and thus the overall quality of the academic program (Cole and Thompson, 2002). According to Andelt et al. (1997), many of the "hot markets" and profitable careers students expect upon graduation are not accessible because "college graduates are unprepared to fulfill the needs of their employers." Furthermore, they contend that educating students for a career in agriculture/natural resources demands not only greater technical skills, but also a more holistic perspective on interaction with society (Andelt et al., 1997).

The Crop, Soil, and Environmental Sciences (CSES) Department at the University of Arkansas currently offers undergraduate Bachelor of Science (B.S.) majors

in Crop Management (CPMG) and Environmental, Soil, and Water Science (ESWS). Previous efforts by the University of Arkansas CSES Department to evaluate academic programs and their effectiveness in terms of graduate employment and earning potential have included alumni surveys and senior exit interviews. Although the department continues to conduct exit interviews with graduating seniors, the last departmental alumni survey was distributed in 1988 (Davis et al., 1991), prior to the establishment of the ESWS degree and Crop Management revisions.

Therefore, as part of the CSES Department's current assessment, two surveys were designed and distributed to alumni and employers in the summer of 2002. The CSES Alumni Satisfaction Survey was sent to 792 departmental alumni, including graduates of the past Agronomy program as well as the current Crop, Soil, and Environmental Science programs. Goals of the alumni survey included assessment of advance degree profitability in terms of earning potential and to determine sectors of the workforce actively recruiting and employing department graduates. The alumni survey was designed with the following specific objective: to obtain demographic, education, and career information. In addition, a comparison of past graduate salaries and opinions regarding employment with those of more recent alumni (1992-2002) will provide important comparative information of students from the past Agronomy Department to the restructured Crop, Soil, and Environmental Sciences Department. The CSES Employer Survey was distributed to 281 environmental and crop science professionals representing various sectors of the workforce including government agencies, private firms, and academic institutions. The employer survey was designed with the following specific objectives: 1) to evaluate the importance of selection criteria for employee hiring, 2) to obtain job descriptions and earning potentials, and 3) to recruit employers interested in interacting with the CSES Department at the University of Arkansas.

MATERIALS AND METHODS

Survey Construction

A survey format was followed as presented by Sheatsley in the Handbook of Survey Research (1983) consisting primarily of closed questions such as multiple choice, rank, rate, and categorical questions. The survey was two pages, front and back, and consisted of only six open-answer questions. Open-answer questions were kept to a minimum due to their ability to elicit repetitious and often irrelevant material (Sheatsley, 1983). Data from three of the CSES Alumni Satisfaction sections will be reported here: Demographics, Educational Background, and Employment. Data from two of the CSES Employer Survey sections, Company Information and Student Advising, will also be included.

Survey Disbursement and Response Rates

The CSES Alumni Satisfaction Survey was distributed in August of 2002 to 792 alumni as an insert in the CSES Department's annual newsletter. Repeated efforts were made encouraging alumni to return completed surveys. In September 2002, an on-line address was created whereby respondents could conveniently complete and submit the survey electronically. Survey responses were received electronically and by mail through December 2002. One hundred and three alumni completed and returned surveys, resulting in a 13% response rate. Thirty-nine responses were received electronically and 64 by mail. Seventy-five percent of those who submitted their survey electronically were post-1992 graduates.

A contact list for the CSES Employer Survey was developed in resemblance to similar surveys administered by other agricultural colleges (Bekkum, 1997; Cole and Thompson, 2002). For example, former and current employers of department graduates, a national list of soybean board members, and the membership list for the Arkansas Environmental Federation were used to garner a potential contact list. Recipients represented various sectors of the workforce including education, local and state governmental agencies, as well as public and private industries. Fifty-three percent of employer survey recipients were located in Arkansas, with the remaining 47% generally located in surrounding states and other states such as Iowa, Indiana, and Illinois. All employers who did not respond within a month of the initial disbursement were contacted again by letter or email. An on-line address was specified where the respondents could opt to complete and submit the survey electronically.

Thirty-one completed employer surveys were received from an initial sample size of 281, resulting in an 11% response rate. Surveys of similar length and content have documented response rates in this area, ranging from 5% to 20% (Wolf and Schaffner, 2000). The relatively low percentage of responses could be an indication that employers who did respond are more interested in educational quality than most, thereby indicating a response bias in the dataset (Wolf and Schaffner, 2000). Over half of the respondents completed the optional section volunteering to interact/collaborate with the department, further supporting the idea that respondents had heightened interest in the quality of higher education.

Statistical Analysis

Frequencies, relative sample abundances, means, ranges, and standard deviations were calculated depending on question type. For some questions, respondents were asked to rate their agreement with statements on a scale from 1 to 5, 5 being "strongly agree" and 1 being "strongly disagree." In these situations, responses above 3 were considered positive and below 3 were considered negative.

RESULTS AND DISCUSSION

Demographics and Educational Background

Despite the fact that the department has changed its name and degree programs over the past 15 years, the demographics of survey respondents were similar to alumni survey respondents in 1988. Eighty-one percent of current survey respondents were male and 97% were U.S. citizens. In 1988, 85% of alumni respondents were male and 95% were U.S. citizens (Davis et al., 1991).

A lower percentage of alumni responding to the 2002 survey (75%) obtained advanced degrees as compared to 85% of those who completed the 1988 survey. The high number of respondents holding advanced degrees is interesting to note given that, during exit interviews, 67% of graduating seniors did not plan to attend graduate school, but rather expected to secure employment immediately upon graduation (Bacon et al., 2000). Bachelor of Science graduates may decide to continue their education, seeking an advanced degree, after working for a period. Of the 63 respondents who received their B.S. at the University of Arkansas, 71% obtained a M.S. and most of these students stayed at the University of Arkansas. Thirty-two percent of these B.S. graduates also went on for a Ph.D., but only 8% received their Ph.D. from the University of Arkansas. Thirty-eight percent of alumni respondents graduated in the past 10 years. Of this group, 44% obtained a M.S. and 10% received a Ph.D. Given the relatively small percentage of alumni who responded to the survey, the high percentage of advanced degree respondents may not be indicative of the total alumni population. Advanced degree holders may be more likely to complete and return the survey, and could represent a bias in survey responses.

Employment

Prospective students frequently question the department recruiter and faculty about potential career opportunities and earnings. To gather this data, alumni and employer respondents were asked about their employment and to disclose salary information. For a first job following graduation, 35% of CSES alumni began working in industry, 36% entered educational positions (most of these were attending graduate school), and 22% obtained jobs with governmental organizations (Table 1). Of those who entered industry positions, 4% were consultants, 13% went into sales, and the remainder held science-related positions such as technicians, field scientists, and researchers. Very few CSES alumni were self-employed. Of the 5% that indicated that they were self-employed, 4% were farmers.

Table 1. Percentage of Crop, Soil and Environmental Sciences alumni employed in different areas following graduation (first job) and in current positions (current jobs).

Area of employment	First job (%)	Current job (%)
Government	22	14
Industry	35	32
Education	36	38
Self	5	6
Other	1	1
Retired	0	9

Alumni were also asked to reveal their current job titles. There was a similar distribution among areas of employment for alumni, except for a notable decrease in governmental positions and the establishment of a new category for those who have retired (Table 1). Current positions differed from initial positions within higher education, as most current job titles had to do with faculty or administrative positions rather than graduate degree-seeking positions. In industry, most job titles were associated with management and executive positions, rather than sales and technician level positions.

Alumni career paths appear to have changed since the 1988 survey. Results from the current survey showed fewer alumni were working in government or were self-employed, and a higher number of respondents went into industry. Factors such as a burgeoning regional population, government downsizing, an influx of industry into the region, as well as the general consolidation of agriculture may all have contributed to the evolving job market. A significant change in the CSES Department's curricula and focus may have also been a factor influencing subsequent career decisions of alumni.

Starting annual salaries were tabulated only for those respondents having received their most advanced degree in the past 10 years (1992 to 2002). Starting salaries for B.S. respondents averaged $23,140, approximately $8,000 less than M.S. respondents (Table 2). As expected, Ph.D. respondents reported the highest average start-

Table 2. Annual starting salaries for Crop, Soil and Environmental Sciences alumni graduating between 1992 and 2002.

Salary information	Alumni with B.S.	Alumni with M.S.	Alumni with Ph.D.
Average	$23,140	$31,290	$42,670
Standard deviation	$7080	$9320	$16,010
Minimum	$13,000	$14,500	$27,000
Maximum	$38,000	$46,000	$59,000
Sample size	18	17	3

ing salary at $42,670. Salaries from the 2002 survey were compared to salaries from the 1988 survey to determine if current graduates are financially "better off" as compared to their peers of 15 years ago. Based on an average annual inflation rate of 3.1% (Annualized Inflation Rates, 2003), starting salaries reported in 1988 would be equivalent to $30,800, $36,800, and $50,300 for B.S., M.S., and Ph.D. degree holders, respectively. The average starting salary for alumni graduating since 1992 was $28,330 (n = 38), after four individuals earning less than $5000 were removed from the dataset. This amount is similar to the average starting salary of $28,190 as reported in the 1988 survey (Davis et al., 1991). However, when adjusted for an average annual inflation rate of 3.10%, that salary in 1988 would be equivalent to $44,560 today, indicating that average starting salaries for our alumni have not kept up with inflation (Annualized Inflation Rates, 2003). However, compared to what was reported in the CSES Employer Survey, salaries appear to be within range of what can be expected in this region. Employers indicated that starting salaries at their places of employment ranged from less than $20,000 to nearly $60,000, with 72% of employers indicating initial starting salaries in the $20,000-$39,999 range. Twenty-one percent of employer respondents, primarily individuals representing the industry sector, indicated starting salaries were in the $40,000 to 59,999 range.

Salaries were categorized with respect to the highest degree earned in part to determine the profitability of investment in an advanced degree. Current salaries ranged from less than $30,000 to $129,999 for all alumni (Table 3). Alumni were not questioned as to the number of years they have been employed in their current position; therefore, salary ranges simply indicate a composite of the total response group. The number of alumni reporting higher-end salaries increased to a greater extent among those with M.S. and Ph.D. degrees (Table 3). For example, only 4% of B.S. respondents, compared to 17% of M.S. and 29% of Ph.D. respondents, indicated they earned annual salaries greater than $80,000.

Table 3. Percentage of Crop, Soil and Environmental Sciences alumni earning salaries within specified ranges (n = 24 for B.S., 35 for M.S., and 31 for Ph.D.).

Salary Category	% Alumni with B.S.	% Alumni with M.S.	% Alumni with Ph.D.
Less than $30,000	25	28.5	10
$30,000-49,999	42	28.5	19
$50,000-79,999	29	26	42
$80,000-99,999	0	14	19
$100,000-129,999	4	3	10

Therefore, not only were starting salaries higher for advanced degree holders, but it also held true that those with M.S. and Ph.D. degrees had the greatest potential to advance their salaries. Current salaries disclosed in the employer survey also ranged from $30,000 to upwards of $100,000 (data not shown), again indicating that alumni salaries are representative of those in this region.

Alumni and employers were asked their opinions regarding whether a B.S. degree is sufficient for a successful career. While alumni felt that one could be successful with a B.S. in Environmental, Soil and Water science (average value of 3.2; where values greater than 3 were considered positive), they were less inclined to agree that a B.S. was sufficient for students majoring in Crop Management (average value of 2.9). This supports the observation from salary data that one can obtain employment with adequate salaries, but potential for advancement is limited with only a B.S. degree. Employers were more likely than alumni to view a B.S. as sufficient for a successful career by assigning values of 3.4 for ESWS and 3.6 for CPMG majors.

Survey participants were asked to indicate their opinions with regard to future job prospects. Most alumni obtained their first job after graduation within a short period. Sixty-one percent of respondents indicated that they obtained their first job immediately after or even before the completion of their last degree and only 6% reported needing more than a year to find a suitable position (n = 89, data not shown). Alumni agreed that jobs should be available in the future for ESWS graduates (average agreement value of 3.9), and were also optimistic about positions related to CPMG majors (average agreement value of 3.5). However, some participants did comment in the surveys that consolidation and downsizing in agronomic fields is a concern. Employers were also generally positive, but more likely to encourage a degree in ESWS (average value 3.7) as compared to a CPMG degree (average value 3.0). Despite the positive responses, 2002 respondents seemed to be slightly less optimistic than 15 years ago when approximately 82% of 1988 respondents indicated that they felt a departmental graduate would be "able to find jobs in the next 4 to 6 years" (Davis et al., 1991). In conjunction with this trend, 71% of respondents in the employer survey indicated that hiring had not occurred at their place of business in over a year. Low employee turnover and low demand were the only two explanations provided for the low hiring rate.

Conclusions

Based on alumni and employer responses, CSES graduates are employed quickly and appear to be earn-

ing competitive salaries. As expected, Ph.D. respondents reported the highest average starting salary, almost $20,000 more per year than B.S. graduates. Furthermore, the number of alumni reporting higher-end salaries increased to a greater extent among M.S. and Ph.D. graduates, thereby affirming the profitability of obtaining an advanced degree. Although the CSES Department at the University of Arkansas has undergone significant changes over the past 15 years, little has changed with regard to student demographics. However, alumni career paths are continuing to evolve, possibly in response to departmental changes as well as regional changes in the workforce. Responses from both alumni and employers have provided valuable insight for improvement of student advising and recruitment. Continual program and graduate evaluation is imperative for graduating competent, competitive, and satisfied individuals.

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Effect of *Triticum turgidum* cytoplasm on test weight of soft red winter wheat

Juan Mayta[*], *Robert Bacon*[†], *John Kelly*[§], *and Edward Gbur*[∞]

ABSTRACT

The effect of *Triticum turgidum* cytoplasm on soft red winter wheat (*T. aestivum*) was studied relative to test weight (bushel weight) and agronomic traits such as heading date, resistance to leaf rust, and plant color. The cytoplasmic effects were studied in three genetic backgrounds by crossing the cultivars Jackson, Pioneer 2684, and Wakefield with a plant introduction PI590277 that carried *T. turgidum* cytoplasm. Twelve alloplasmic and euplasmic populations were developed through a backcrossing procedure. The study used a micro test weight procedure to compare F_4 lines within the populations for test weight. The data for other agronomic traits were taken by field ratings. Statistical analysis showed no significant gain in test weight due to *T. turgidum* cytoplasm (P=0.32). However, an important effect on leaf-rust reaction was observed. The lines derived from 'Pioneer 2684' and 'Wakefield' that carried *T. turgidum* cytoplasm were more resistant to leaf rust than were their respective euplasmic lines. Euplasmic and alloplasmic lines derived from 'Jackson' did not show difference in leaf-rust reaction. A yellow color at harvest and late heading date were observed on alloplasmic lines derived from 'Pioneer 2684' and 'Wakefield' cultivars. The third cultivar 'Jackson' showed no yellow coloration at harvest.

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MEET THE STUDENT-AUTHOR

Juan Mayta

I graduated in May 2003 with a major in crop science and a minor in agricultural business. I am originally from Bolivia. I had the opportunity to get an associate degree in agronomy at the Pan American School of Agriculture "Zamorano" in Honduras. I transferred to the University of Arkansas in 2001. I was awarded the Foundation for International Exchange of Student (FIES) Scholarship. I was also recipient of the World Hunger Scholarship from the Methodist Church and the Fontaine Richard Earle Scholarship, which helped me to accomplish my goals. My two years at the University of Arkansas have been an excellent experience not only academically but also in my personal life.

From the beginning I was able to gain hands-on research experience, first through my job with the Wheat Breeding Program getting experience in cultivar development of wheat. I was also able to enroll in the Honors Program of the Department of Crop, Soil and Environmental Sciences, which I think helped me to get the most from the college experience not only by learning research techniques but also by allowing me to grow as an individual. I was also an active member of the Agronomy Club in my department, for which I served as a treasurer. By getting involved with the Agronomy Club I had the opportunity to get acquainted with the faculty and make good friends. I pursued my undergraduate research as a requirement for the Honors Program and also had the opportunity to present my undergraduate research at the American Society of Agronomy Meeting in Indianapolis in 2002. I will continue my graduate studies at the University of Arkansas working with the Soybean Breeding Program. My long-term goals are to go back to Latin America and be able to improve food production in our countries. I would also like to teach at some point and share the great experience I had in the U.S.

INTRODUCTION

Genes from wild relatives of common crops can have desirable effects on agronomic characteristics. It is possible to introduce cytoplasmic variability to broaden the genetic base; this resulting genetic base can be used for breeding desirable characteristics. The wild and cultivated relatives of common wheat (*Triticum aestivum*) that produce fertile alloplasmic lines may provide a large reservoir of cytoplasmic variability for this crop (Kofoid and Maan, 1981).

The weight of a volume of seed (test weight) is an important characteristic of cereals. Test weight is economically important for wheat growers as well as for industrial users of wheat. Wheat is traded on the basis of USDA #2 standard, which among other things has a minimum requirement for test weight of 746 kg/m3 (58 lb/bu). Farmers that produce lower test-weight wheat will receive lower prices. Test weight is used because it is considered to be a predictor of flour yield; thus, millers are also concerned about this trait. Another important consideration is transportation and storage of grain. Costs are lower if millers can transport and store more weight in less space (Schuler, 1992). Therefore, improvements in this characteristic benefit the farmers and the wheat industry in general.

Schuler (1994) indicated that cultivars differ in test weight when grown under similar conditions. This difference indicates a genetic component, and therefore, a potential for improvement of test weight through plant breeding.

The Agricultural Research Service, U.S. Department of Agriculture and the Agricultural Research Center of Washington State University reported that *T. turgidum*

had a positive effect on test weight of 'Stephens', a soft white winter wheat cultivar (Allan, 1997). Although research has shown alien cytoplasm effects when introduced into specific cultivars, each breeding program must test adapted germplasm in its local region to see if gains in traits can be uncovered and exploited locally. The objective of this research was to determine the effect on test weight and other agronomic traits contributed by *T. turgidum* cytoplasm introduced into three soft red winter wheat backgrounds.

MATERIALS AND METHODS

The adapted cultivars Jackson, Pioneer 2684, and Wakefield and the plant introduction with the alien cytoplasm (PI590277) from *T. turgidum* were used in this study. Since the cytoplasm is maternally inherited, reciprocal crosses were made using PI590277 as both the male and female parent. The resulting F_1 plants had 50% of their nuclear genes from each parent. A backcross was made in order to produce plants with 75% of their nuclear genes from the adapted cultivars. As a result two populations were created: one alloplasmic having the alien *T. turgidum* cytoplasm, and one euplasmic having the conventional *T. aestivum* cytoplasm. Additionally, both populations had 75% of the nuclear genes derived from the cultivars. These populations were allowed to self-pollinate until the F_4 generation.

Seeds threshed from single F_4 spikes were planted on 5 Oct. 2001. The seeds were planted 0.4 cm apart in blocks 1.5 m wide with four 1.8 m-long plots. The plots were planted at the University of Arkansas Research and Extension Center, Fayetteville, on a Captina silt loam. For weed control, "Hoelon" was applied at a rate of 1.5 L/ha on 7 Nov. 2001. Additionally, 112 kg/ha nitrogen were applied on 15 Mar. 2002. The total number of plots was 960 with 160 plots for each of the six crosses.

The six populations were divided in two groups of 80 plots in order to have replications for statistical analysis. The design was a randomized complete block with two replications in a 3x2 factorial. The characteristics measured for each individual plot were test weight, days to heading, and leaf rust reaction. A contingency table was constructed to compare differences in leaf rust reaction. Histograms were used to compare days to heading. Color at harvest was observed but was not subjected to formal statistical analysis.

Test weight

Four spikes per plot were harvested. The spikes were threshed and cleaned in a wheat single-spike thresher (Precision Machine Company, Inc. Lincoln, Neb.). The seeds from three spikes were bulked in order to measure their weight in a 4.5 ml cylinder. This micro test weight per volume of 4.5 ml was then expressed in kilograms per cubic meter and also in its equivalent in pounds per bushels. This procedure reported by Schuler (1992) was developed by the wheat breeding program at the University of Arkansas.

Days to heading

Days to heading were considered as the number of days from 1 Jan. until 50% of the spikes emerged from the leaf sheath or boot. The ratings were taken from 24 Apr. through 20 May 2002

Leaf rust assessment

The following scale was used: 0-immune; 1-practically immune; 2-extremely resistant; 3-resistant; 4-moderately resistant; 5-transition of 4 to 6; 6-moderately susceptible; 7-susceptible; 8-transition of 7 to 9; 9-highly susceptible (USDA/ARS Cereal Disease Laboratory at the University of Minnesota, 2003.) The ratings were taken at Feekes stage 10.5, which represents the flowering stage (Large, 1954).

RESULTS AND DISCUSSION

Test weight

Among the three genetic backgrounds the alloplasmic and euplasmic lines were almost identical for the 'Jackson' and 'Wakefield' populations (Table 1). The alloplasmic and euplasmic lines derived from 'Pioneer 2684' showed the biggest difference (10 kg/m^3); however, this difference was not statistically significant. The effect of *T. turgidum* cytoplasm on average test weight was not statistically significant by itself (P=0.32) or in its interaction with cultivar (P=0.46).

Heading date

The effect of cytoplasm was different for heading date for each cultivar. Lines derived from 'Jackson' did not show variation between alloplasmic and euplasmic lines; both populations averaged 121 d to heading. Although there were no differences between the euplasmic and alloplasmic lines derived from 'Jackson,' the earliest heading date in our study (116 d) was among the euplasmic lines using 'Jackson' as a parent (Fig. 1). Lines derived from 'Pioneer 2684' and 'Wakefield' both showed differences among their respective alloplasmic and euplasmic lines; the data distribution for alloplasmic lines derived from 'Pioneer 2684' showed a group of lines with an average days to heading of 121 d, and another group with an average of 137 d. Likewise, the data distribution for lines derived from 'Wakefield' showed a group of lines with an average number of days to heading of 122 d and another group with an average of 138 d (Fig. 1). The alloplasmic lines derived from Wakefield were the latest to reach the heading stage.

Table 1. Test weights of alloplasmic and euplasmic lines derived from three genetic backgrounds of soft red winter wheat.

Cultivar	Cytoplasm	Test-weight
		kg/m³ (lb/bu)
Jackson	Euplasmic	694 (54.0)
	Alloplasmic	692 (53.8
Pioneer 2684	Euplasmic	664 (51.6)
	Alloplasmic	654 (50.8)
Wakefield	Euplasmic	682 (53.0)
	Alloplasmic	680 (52.8)
		NS[1]

[1] NS = non-significant (P<0.05)

Leaf rust assessment

Leaf rust reaction assessment showed differences between and among the different populations; for instance, the alloplasmic lines derived from 'Jackson' showed more susceptible plants than the euplasmic lines derived from 'Jackson' (Fig. 2). On the contrary, 'Pioneer 2684' showed more resistant plants among the alloplasmic lines (99 out of 160). 'Wakefield' lines showed the largest number of resistant lines among its alloplasmic population (122 out of 160).

Color

An interesting characteristic that was observed was the color of the plants at maturity. The alloplasmic lines with a genetic background from 'Pioneer 2684' or 'Wakefield' both showed more yellow color at maturity than the other wheat lines. The effect was more consistent among lines derived from 'Wakefield' (data not shown). There were no coloration differences between euplasmic and alloplasmic lines derived from 'Jackson'. all the lines had the traditional wheat color at maturity.

Effects of alien cytoplasm were not consistent among the genetic backgrounds. The results indicated a nuclear-cytoplasmic interaction. According to Kofoid and Maan (1981) the mode of inheritance and interrelations between maternal parents and cytoplasmic and nuclear genetic controls are not well understood, and more research is needed.

Triticum turgidum cytoplasm did not show a positive effect on test weight of lines derived from the three cultivars of soft red winter wheat. Although the effect on test weight was not significant in our study, it might be possible to select individual lines within the populations that have higher test weights. As Schuler (1992) points out, packing efficiency, kernel size, and shape should be evaluated. Also spike characteristics such as spike density, number of spikelets per spike, and the number of fertile florets per spikelet should also be considered. A study evaluating these variables could lead to a better understanding of the effect of cytoplasm on test weight

The difference in leaf-rust reaction was more significant among the alloplasmic lines of 'Pioneer 2684', and 'Wakefield'. For leaf rust reaction, it is possible that each cultivar had resistance genes for leaf rust; however, since there was variation among the different lines for each cross, the data indicated an effect of the alien cytoplasm. This result was particularly true with the 'Pioneer 2684' and 'Wakefield' populations, which showed the highest number of resistant lines. Additionally, it is important to point out that the lines that had the highest leaf-rust reaction among the alloplasmic lines of 'Pioneer 2684' and 'Wakefield' also had late heading dates. These data indicate the possibility of linkage between leaf-rust resistance and late heading date; however, there were exceptions among these lines, with some lines having an average to early heading date.

The bimodal distribution of days to heading for the alloplasmic lines derived from 'Pioneer 2684' and 'Wakefield' is another example of nuclear-cytoplasmic interactions (Fig. 1). The distribution of the data was divided into two groups; one group with number of days to heading as early as 118 d, and as late as 127 d; the second group of lines had the latest number of days to heading having a range from 137 to 140 d.

Regarding the color of the spikes at harvest, the alloplasmic lines of 'Wakefield' and 'Pioneer 2684' showed a more yellow coloration than typical wheat. Although in our study we did not take measurements on this variable, it can be subject of further research studying cytoplasmic effects.

ACKNOWLEDGMENTS

Appreciation is extended to the Dale Bumpers College of Agricultural, Food and Life Sciences Undergraduate Research Award Program and the Arkansas Agricultural Experiment Station for providing financial support.

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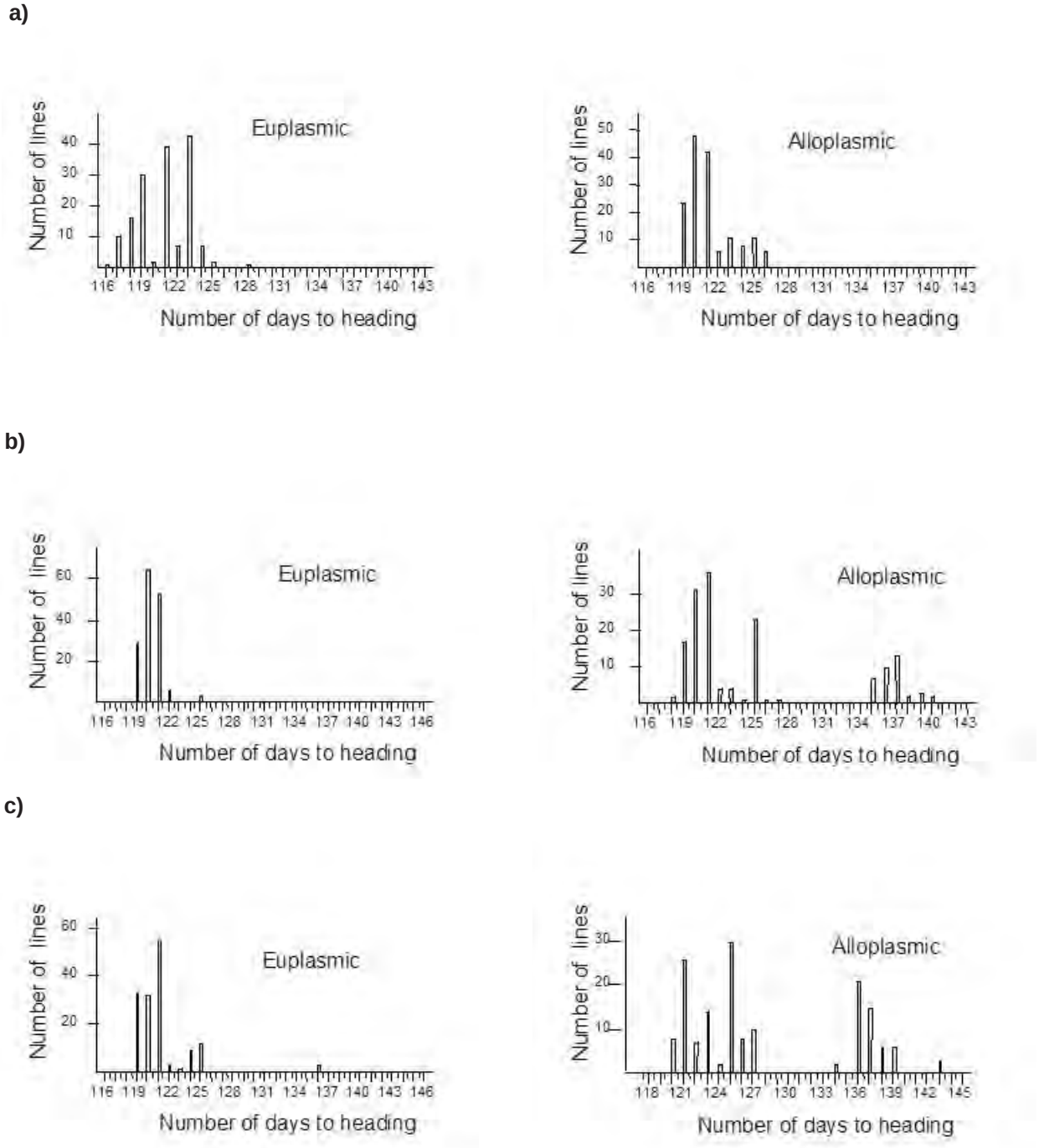

Fig. 1. Distribution of number of days to heading (x-axis): comparing euplasmic and alloplasmic populations. a) 'Jackson', b) 'Pioneer 2684', c) 'Wakefield'.

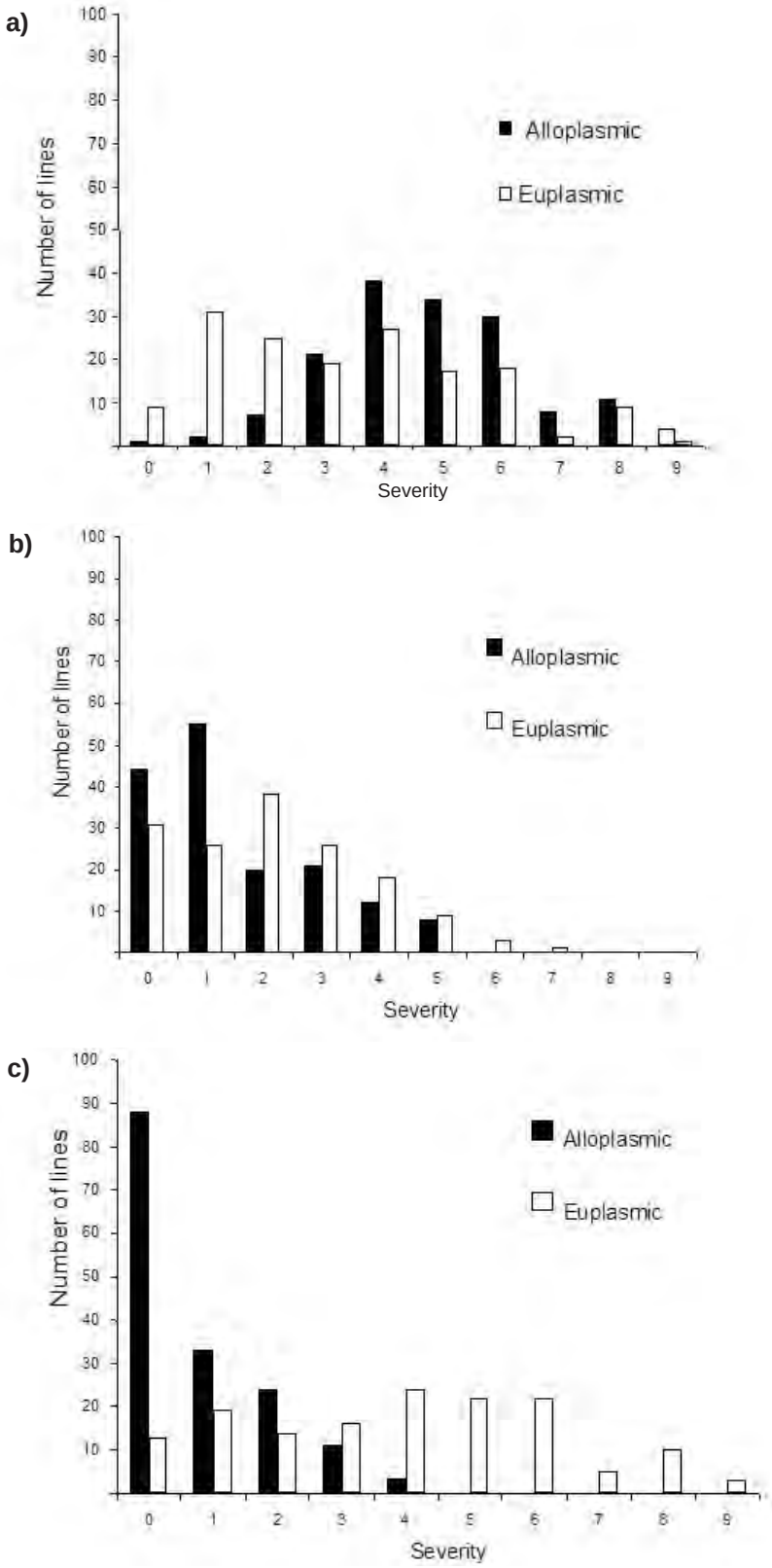

Fig. 2. Leaf rust reaction among alloplasmic and euplasmic lines derived from: a) 'Jackson', b) 'Pioneer 2684', and c) 'Wakefield'.

The effects of potential organic apple fruit thinners on gas exchange and growth of model apple trees: A model plant study of transient photosynthetic inhibitors and their effect on physiology and growth

J. D. McAfee[*] *and C. R. Rom*[†]

ABSTRACT

Few fruit thinners have been certified for organic fruit growers. Previous studies have shown that herbicides or shade are capable of reducing photosynthesis and are effective fruit-thinning techniques, although impractical. This project evaluated use of a model plant system of vegetative apple trees grown under controlled conditions to study photosynthetic inhibitors, which could be used as potential organic thinning agents. Various concentrations of osmotics, salts, and oils (lime-sulfur, potassium bisulfite, potassium bicarbonate, sodium chloride, soybean oil) were applied to actively growing apple trees and showed a reduced trend on the rate of apple tree photosynthetic assimilation (Pn), evapotranspiration (Et), and stomatal conductance (gs). From two studies, it was observed that treatments of 2% lime-sulfur (LS) + 2% soybean oil (SO), 4% SO, 8% LS, 5% potassium bicarbonate ($KHCO_3$), and 5% potassium bisulfite ($KHSO_4$) all significantly reduced Pn. The 4% LS + 2% SO, 4% LS + 4% SO, 0.5% sodium chloride (NaCl), and 2% NaCl did not significantly reduce Pn. The response of Et was significantly reduced by 2% LS + 2% SO, 5% $KHCO_3$, and 4% SO. In a second study, trees had reduced Pn, Et, and gs after the application of 4% LS + 4% SO, 2% NaCl, 5% $KHCO_3$, and 5% $KHSO_4$. Stem weight, total plant weight, average leaf weight, and leaf surface area of the treated plants, although reduced, were not significantly so when compared to the control 20 d after treatment.

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[†] Curt R. Rom is an associate professor in the Department of Horticulture.

MEET THE STUDENT-AUTHOR

Jason D. McAfee

I graduated from Harrison High School in 1997. I began a career at the University of Arkansas in 1998. I did not enter the Horticulture Department until the following year. Since I have been in this department, I have found numerous opportunities in the area of plant science.

As an undergraduate in the Department of Horticulture, I decided to pursue my M.S. degree in the area of fruit crops. To give me some experience in the research field, I was fortunate to receive some grant money through the Mitchener Family Undergraduate Research Award. Dr. Curt Rom advised and encouraged me to do this research project to help prepare me and give me experience for graduate school. I have learned that research can be a very tedious task at times, but continuous persistence can be rewarding in the end.

I am proud to say this research won first place for the undergraduate presentation at the 63rd Annual Meeting of the Southern Regional American Society for Horticultural Science at Mobile, Ala. I would like to express my thanks and appreciation to Dr. Curt Rom for his cooperation and support.

INTRODUCTION

Fruit thinning is a technique essential to apple production that ensures fruit quality by maximizing fruit size and sustaining the tree's potential to annually bear marketable fruit. A number of chemical treatments are available to conventional apple growers but few are currently registered or recommended to certified organic growers. Organic growers typically rely on mechanical removal of excessive fruitlets by hand labor, which is very expensive. In order to test potential organic fruit-thinning treatments and develop reliable, economical thinning technologies, it is necessary to create a model system to test naturally occurring and organically certifiable compounds.

Over the past four decades, research has demonstrated the value and appropriate timing of thinning techniques to maximize fruit size and flower bud development for the following year's crop. Early studies showed the correlation between factors such as shade and the reduction in photosynthesis and how they affect fruit growth (Heinecke, 1966). Currently, fruit thinning is accomplished through synthetic plant-growth regulators, herbicides, and caustic chemicals or by mechanical means (hand removal of flowers or fruitlets). Due to the expense of hand removal of flowers and fruitlets, most fruit-thinning research has focused on chemical methods of application. Past studies have shown that herbicides such as terbacil and increased shade are good fruit-thinning techniques (Byers et al.,1990). Furthermore, the primary focus of research has been on chemical treatments for conventional orchards. Several of these chemical products have been registered for fruit-thinning purposes. However, none of these chemicals are certified for organic fruit producers.

The present increase in market demand for organically grown food has increased the need for science-based technologies that are certifiable organic alternatives to conventional methods and hand labors. It was proposed that some certified organic spray materials may cause a transient suppression or inhibition of photosynthesis. The reduction of carbohydrate supply caused by this suppression would result in strong inter-fruit competition for metabolites whereby smaller or developmentally delayed fruit would not compete and therefore abort. A model plant test under a controlled environment of treatment effects on photosynthesis and vegetative growth may indicate the usefulness of such materials for fruit thinning in the field. The objective of this project was to study the effects of potential organic thinning agents on gas exchange and growth of vegetative apple trees as a model system.

MATERIALS AND METHODS

Study 1 (February – March, 2002).

'Golden Delicious'/M.7a nursery stock trees (approximately 0.5-0.75 cm diameter) were potted in 7.6 L pots with a soil medium consisting of a Sunshine SB500 mix (35-45% bark, Canadian sphagnum peat moss, vermiculite, perlite, dolomitic limestone, gypsum, starter nutrient charge, and wetting agent) at the University of Arkansas Agricultural Research and Extension Center, Fayetteville, in January, 2002. At potting, trees were cut 2.5 cm above the graft union and subsequently new growth was trained to single shoot and all lateral buds were removed. Plants were grown in a greenhouse with temperatures of 25-30/18-20°C (day/night). Trees were watered as needed and fertilized with a weekly Peters' soluble 10N-4.4P-8.3K fertilizer. Pests were controlled by chemical treatments only if found present from scouting.

Trees were divided into six replications of 10 single-tree experimental unit treatments. When shoots were approximately 20 cm in height (Feburary, 2002) treatments were applied one time for the study. Treatments included: 1) 4% soybean oil (SO); 2) 2% lime-sulfur (LS) + 2% SO; 3) 4% LS + 2% SO; 4) 4% LS + 4% SO; 5) 0.5% sodium chloride (NaCl); 6) 2% NaCl; 7) 5% potassium bicarbonate ($KHCO_3$); 8) 5% potassium bisulfate ($KHSO_4$); 9) 8% LS; and 10) untreated control. Treatments were applied once with 1 L spray bottles until leaves were dripping. Trees were placed in a completely randomized design in the greenhouse.

Study 2 (November – December, 2002).

M.111 EMLA clonal apple-rootstock liners (0.30-0.50 cm diameter) were planted in 4.1 L pots with Sunshine SB500 mix and grown in a greenhouse (as described previously) in late August, 2002. After planting, liners were cut 2.5 cm above the soil leaving two buds exposed. Plants were grown as single shoots and managed as described above. Treatments included: 1) LS 4% + SO 4%; 2) 2% NaCl; 3) 5% $KHCO_3$; 4) 5% ($KHSO_4$); and 5) untreated control. The trees were placed in a completely randomized design with five single-tree experimental unit replications of each treatment.

Measurement Variables. A CIRAS-1 differential CO_2/H_2O infra-red gas analyzer with integral cuvette air-supply unit and Parkinson leaf cuvette with an automatic light control was used for the measurements of photosynthetic assimilation (Pn), internal CO_2 (Ci), evapotranspiration (Et), conductance (gs), leaf temperature (T), relative humidity (RH), and photosynthetically active radiation (PAR) in each study. The chamber of the leaf cuvette measured a leaf surface area of 2.5 cm^2. The leaf chamber conditions were set for 50% RH, 350 ppm CO_2. Light saturation of PAR for all measurements averaged >1000 $mmol/m^2/s$ and a temperature of 25°C.

Each tree was labeled at the fifth and seventh leaf for continued measurement of the same leaves. In Study 1, leaves were measured on -1, 1, 4, 8, 15, and 22 d after treatment. In Study 2, leaves were measured on -1, 1, 3, 10, and 20 d after treatment. Following measurements, trees were cut off at the graft union. Leaves were divided between treated and newly emerged. Growth measurements were recorded for the stem dry weight (oven dried), total plant dry weight (oven dried), total leaf surface area, and average leaf area. The growth and treatment measurements were based on a previous, similar study using shade treatments (Barden, 1977). The various treatments used in the two studies represented a range of solution-pH and electrical conductivity (EC) characteristics (Table 1). The statistical analysis for this study was done using JMP-IN software and an LSD student's t-test.

RESULTS AND DISCUSSION

Study 1.

Treatments of the 4% SO and 2% LS + 2% SO significantly reduced Pn at 1 and 4 d after treatment (DAT). Treatments of 5% $KHCO_3$, 5% $KHSO_4$, and 8% LS significantly decreased Pn 4 DAT. Three different treatment combinations of LS and SO treatments were introduced to compare differences in concentration. The lowest concentration (2% LS and 2% SO) was the only one to show a significant decrease in Pn when compared to the higher concentrations (Fig. 1A). Treatments of 5% $KHCO_3$, 5% $KHSO_4$, 4% SO, 8% LS, and 2% LS + 2% SO significantly decreased Et 4 DAT (Fig. 1B). Treatments of 5% $KHCO_3$, 5% $KHSO_4$, 4% SO, 8% LS, and 2% LS + 2% SO significantly decreased gs 4 DAT (Fig. 1C).

Study 2.

Treatments of 5% $KHSO_4$, 5% $KHCO_3$, and 2% NaCl significantly reduced Pn 20 DAT (Fig. 2A). Treatments of 5% $KHSO_4$ and 5% $KHCO_3$ significantly reduced Et (Fig. 2B). A treatment of 5% $KHSO_4$ significantly reduced gs 10 DAT. A treatment of 5% $KHCO_3$ significantly reduced gs 20 DAT (Fig. 2C). Stem weight, total plant weight, average leaf weight, and leaf surface area of the treated plants, although reduced, were not significant when compared to the control 20 DAT.

The treatment of $KHSO_4$ had the greatest decrease in Pn out of all treatments. The gs was reduced following treatment. As an acidic salt, this treatment potentially

decreased the number of stomates present on the leaf's surface, which resulted in a decrease of Pn, Et, and gs. The leaves exhibited necrotic burn. This damage appeared as small burned lesions randomly distributed all over the leaves. A 5% concentration of $KHSO_4$ has a pH of 1.1 and a high EC of 333 mV (Table 1); this caustic nature is presumed to be the cause of the necrosis.

The LS treatments have the potential to act as a caustic agent for fruit-thinning purposes. This compound has a pH in the range of 10-11 and a low EC measured at -246, acting as a strong base. When applied as a thinning agent, it is capable of stressing the leaves of the tree due to osmotic tension on stomatal and epidermal cells therefore lowering Pn, Et, and gs.

A treatment of SO may potentially cover and plug stomates. This can slow the Pn of the leaves and results in a lower Et. An SO application on the tree may cover stomatal pores on the leaf surface of the plant. This may decrease the plant's photosynthesis and transpiration and further decrease the amount of carbohydrates available for cell division resulting in fruit abortion (Weller and Ferree, 1978).

This model plant study was a successful method for studying photosynthetic inhibitors under a controlled environment. In both studies, osmotics, salts, and oils reduced Pn, Et, and gs in model vegetative apple trees. Future studies will need an increase in replications to show more significance between treatments and control. The next step is to study each individual photosynthetic inhibitor for differential effects among various concentrations. Once concentration effects have been measured, it will be necessary to apply these photosynthetic inhibitors in the orchard during the post-bloom period. This will show the true potential of these inhibitors as fruit-thinning agents.

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Table 1. Characteristics of spray solutions used in studying effects on apple leaf gas exchange.

Treatment	pH	EC (mV)[2]
Control H_20	6.9	15
Potassium bisulfate 5%	1.1	333
Potassium bicarbonate 5%	8.3	-71
Sodium chloride 0.5%	4.7	140
Sodium chloride 2%	4.9	124
Lime-sulfur 8%	11.3	-246
Soybean oil 4%	9.5	-145
Lime-sulfur 2% + soybean oil 2%	10.4	-198
Lime-sulfur 4% + soybean oil 2%	10.8	-219
Lime-sulfur 4% + soybean oil 4%	10.9	-224

[2] EC = electrical conductivity; mV = millivolts.

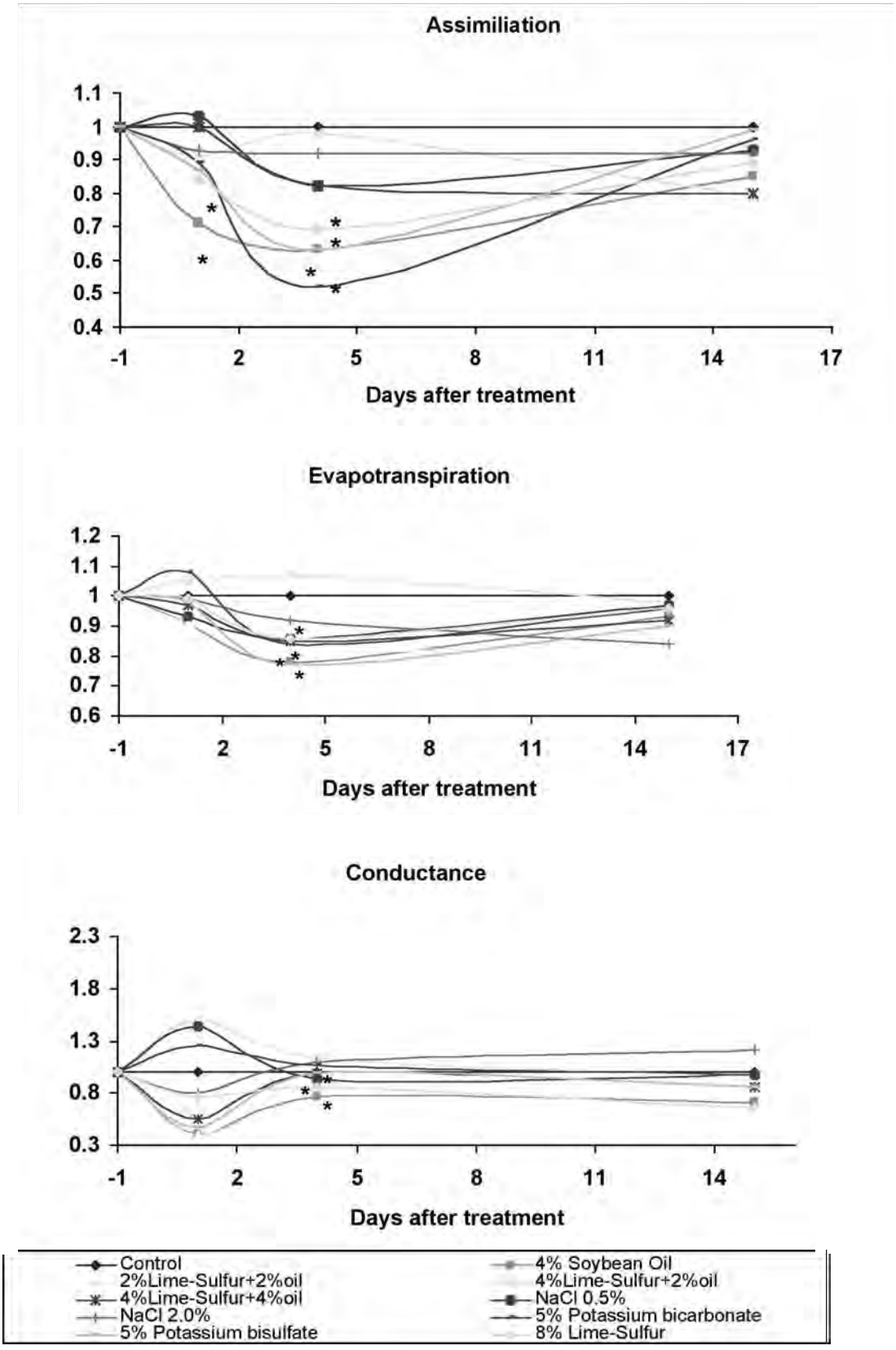

Fig. 1. Pn (A), Et (B), and gs (C) in response to various treatments of organic thinning chemicals, Fayetteville, Ark., February – March, 2002. Mean separation by LSD (P≤0.05, n = 10).

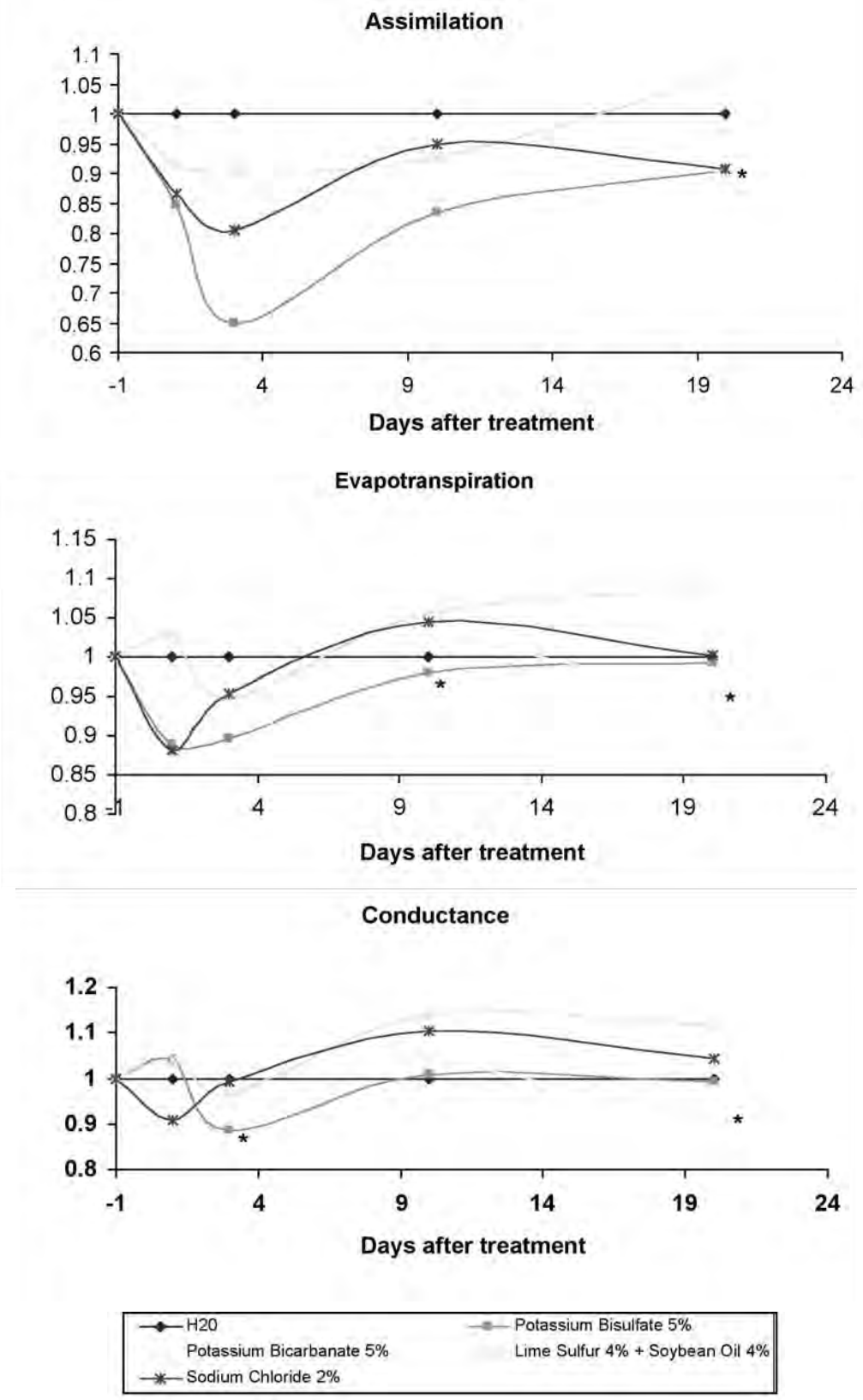

Fig. 2. Pn (A), Et (B), and gs (C) in response to various treatments of organic thinning chemicals, Fayetteville, Ark., November - December, 2002. Mean separation by LSD (P≤0.05, n = 5).

Evaluation of a new extraction system for rapid measurement of surface lipid content of rice for degree of milling estimation

Amanda Parker[*]*, Cynthia Rohrer*[†]*, and Terry Siebenmorgen*[§]

ABSTRACT

The objective of this research was to evaluate a potential time-saving method for surface lipid content (SLC) measurement of milled rice by utilizing new extraction technology. The SLC is often used as the basis for quantifying the degree to which bran has been removed from kernels during the rice milling process; this quality factor is often referred to as degree of milling (DOM). The SLCs of two long-grain cultivars of rice, 'Cypress' and 'Cocodrie', were determined using an accelerated solvent extraction system (ASE) and compared to the conventional, manual system (Soxtec extraction system) that is typically used for SLC measurement. Both systems were tested at extraction temperatures of 115°C, 135°C, and 150°C with total extraction durations of 30 and 50 min. Results indicated that the longer extraction duration, 50-min, produced the lowest SLCs and higher temperatures generally produced lower SLCs. Overall, the surface lipid levels measured by the ASE were similar to or greater than the Soxtec, suggesting that the ASE is as reliable as conventional methods used for DOM determinations based on surface-lipid extractions, with the added advantages of reducing organic solvent usage, extraction time, and labor.

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[†] Cynthia Rohrer is a research associate in the Food Science Department.

[§] Terry Siebenmorgen, faculty sponsor, is a professor in the Food Science Department.

MEET THE STUDENT-AUTHOR

Amanda Parker

I was born in Springfield, Mo., where I graduated from Glendale High School in 2000. I am currently a junior at the University of Arkansas and a food science undergraduate. I am a member of Pi Beta Phi sorority, Gamma Beta Phi, and the National Society of Collegiate Scholars. I received the non-resident tuition award and a food science scholarship to help me pursue my goals. Recently, I received the Silo Undergraduate Research Fellowship to help fund my research.

I plan to graduate in 2004 with a bachelor's degree in food science and a minor in communications. I then want to pursue graduate school to further my education in Food Science. My goal is to work in the field of research and development.

In the Food Science Department, I have been given the opportunity to work in rice research. I decided to do this project upon the encouragement of Dr. Cindy Rohrer and Dr. Terry Siebenmorgan who both have been a tremendous help. Through my research I learned many things about degree of milling and surface-lipid content of rice. This opportunity has also helped me improve my laboratory and research skills. This has been a great experience that I feel will assist me in the future.

INTRODUCTION

Milling is a mechanical process during which brown rice is subjected to abrasive or frictional action to remove the germ and bran layers to yield white rice. Due to the high content of oil in bran (up to 24% oil), bran remaining on the kernel after milling can result in off-flavors and odors from oil oxidation. Therefore, it is important that rice be milled sufficiently to remove bran to acceptable levels. The degree of milling (DOM) of rice is the extent to which bran has been removed from rice-kernel surfaces and is important in determining head rice yield (Sun and Siebenmorgen, 1993); viscosity (Perdon et al., 2001); starch gelatinization (Marshall, 1992); and sensory quality (Piggott et al., 1991).

Several methods have been used to estimate DOM, including visual examination, optical measurements, staining techniques, and chemical composition analysis. One commonly used technique for chemical composition analysis is to measure the amount of lipids remaining on the surface of the rice kernel through a petroleum-ether extraction (Watson et al., 1975). A widely used method of petroleum-ether lipid extraction is the conventional Soxtec extraction system. This system is an improvement over previously used systems, such as the Soxhlet system, in terms of saving time, solvent, and labor. For example, the Soxtec extraction system has been found to be as accurate as the conventional Soxhlet system in lipid extraction (Morrison, 1990) with the advantages of reduced extraction durations (often less than 1 h), and lower solvent levels (usually less than 50 mL/sample) compared to the Soxhlet system.

One limitation of the Soxtec manual system is that it requires the presence of the user for manual operation of the lever arm in order for the rinsing step to be completed during extraction. With the increased demand for fast, accurate DOM measurements, alternative methods that are less labor-intensive, use less solvent, are capable of more samples per day, and are more automated than the Soxhlet or Soxtec, have attracted interest. One such extraction system, known as pressurized liquid extraction or more commonly by its trade name, accelerated solvent extraction (ASE), has been utilized to extract oil from several different matrices. Through this system, up to 24 samples can be loaded into the instrument, and using elevated temperatures (up to 200°C) and pressures (up to 3000 psi), extractions are performed quickly in only a small quantity of solvent (<50 mL); also, user

presence is not required to perform any operations on the instrument during the entire extraction process. The completion of 24 samples per day, depending on the extraction duration, can be accomplished at an unhurried pace allowing other laboratory tasks to be finished while the samples are extracting. When compared to conventional methods, i.e., the Soxhlet and Soxtec, this fully automated process produces results in a fraction of the time (< 20 min/sample), with final samples prepared in closed collection vials for further clean-up steps or immediate analysis.

Key parameters to obtaining optimal results with the ASE system are extraction temperature, number of static cycles, and static phases. The static cycle allows the sample to be held for a static or stationary time period in contact with fresh solvent during the extraction process, which aids in maintaining a favorable extraction equilibrium. As the temperature is increased, the viscosity of the solvent is reduced, thereby increasing its ability to wet the matrix and solubilize target analytes. Increasing static phases at elevated temperatures allows compounds of interest to diffuse more quickly into the extraction solvent, thereby enhancing extraction efficiency.

In order to assist the rice industry in providing fast, accurate DOM measurements, this study was conducted to evaluate operating conditions of the ASE system for surface-lipid content (SLC) determination. If the parameters discussed above for the ASE are fine-tuned for accurate and reliable measurements compared to the commonly used Soxtec method, the procedure for quantifying SLC could be standardized and automated for laboratory DOM determination.

MATERIALS AND METHODS

Sampling Techniques

Two long-grain rice cultivars, 'Cypress' and 'Cocodrie', were harvested from the Northeast Research and Extension Center, Keiser, Ark., in September 2002 at moisture contents (MCs) of 17.2 and 18.5% (expressed on a fresh-weight basis), respectively. Immediately after harvest, the rice was cleaned using a dockage tester (Model XT4, Carter-Day Co., Minneapolis, Minn.) and gently dried by placing the rice onto screen trays in a controlled temperature and relative humidity chamber (21°C, 53% RH) to achieve approximately 12% MC. Following drying, a sample of 150 g of rough rice from each cultivar was dehulled using a Satake Rice Machine (Type THU, Satake Engineering Co., LTD, Tokyo, Japan). This was repeated five times in order to obtain sufficient head rice (milled kernels > 75% of original kernel length) quantities for the analysis. The resulting brown rice was milled in a laboratory mill (McGill No. 2, RAPSCO, Brookshire, Tex.) for 30 s. Placing a 1.5 kg weight on the lever arm 15 cm from the middle of the mill chamber controlled the pressure on the rice during milling. Head rice was separated and head rice yields (HRYs) were determined using a Grainman shaker table with a 4.76 mm screen size (Grainman Machinery Mfg., Corp., Miami, Fla.). The HRYs for 'Cypress' and 'Cocodrie' were 70% and 64%, respectively. Head rice samples were placed in plastic freezer bags, purged with nitrogen, and stored at –10°C until subsequent extraction with the Soxtec system and ASE. Thirty-six samples per variety were extracted on the Soxtec system and 54 samples were extracted on the ASE per variety for a total of 180 samples analyzed.

Surface Lipid Extraction

Soxtec. Surface lipids were extracted from head rice using a Soxtec Avanti 2055 Manual Extraction unit (Foss Tecator, Eden Prairie, Minn.) with petroleum ether (ACS grade, Mallinckrodt Baker, Paris, Ky.) as the extracting solvent. Samples were pre-dried prior to extraction on both systems by placing 5 g of head rice into cellulose-extraction thimbles (33 mm i.d. x 80 mm, Foss North America, Inc., Eden Prairie, Minn.) with a defatted cotton plug placed on top of the sample to keep the sample from boiling out, and placed in a convection oven at a constant temperature of 100°C for 1 h (Hogan and Deobald, 1961). Petroleum ether (70 mL) was measured into each extraction cup and the thimble was lowered to immerse the sample in the solvent for two treatment conditions of 15- and 25-min boiling durations. The boiling temperature of the solvent was set on the unit so that three different temperatures of 115°C, 135°C, and 150°C were tested. The thimble was then manually raised by the operator above the solvent surface and rinsed for two durations, 15 or 25 min, by the condensed solvent to extract remaining lipids on the surface of the kernels (Chen and Siebenmorgen, 1997). After rinsing, the solvent flow was discontinued by manual operation and any solvent from the extraction cup was evaporated and collected inside the unit for 5 min. The total extraction length was 30 min and 50 min/sample at each of the three temperature settings. The design of the Soxtec system allowed only six samples to be extracted simultaneously. The extraction cups were dried at 100°C for 30 min to remove any residual petroleum ether, leaving only the dry material, which represented the extracted surface lipids. Following drying, the cups were transferred to a desiccator to cool for 30 min, and the weight of the remaining lipids in the cups was used to calculate SLCs by expressing as a percentage of the original head rice (5 g).

Accelerated Solvent Extractor. Extraction of surface lipids from pre-dried head rice, as described above, was

accomplished by the use of an accelerated solvent extractor (ASE 200, Dionex, Sunnyvale, Calif.). The pressure during extraction was maintained at 1500 psi (10,342 KPa) with temperatures of 115°C, 135°C, and 150°C. Each pre-dried head rice sample (5 g) was placed in an extraction cartridge, loaded onto the carousel holder of the ASE, and extracted for the following durations: two 25-min cycles, two 15-min cycles, and one 30-min cycle (using petroleum ether as the extracting solvent) representing total extraction durations per sample of 50 min, 30 min, and 30 min, respectively. The ASE extracts were collected in 40 mL glass vials and comprised approximately 25 mL of petroleum ether and lipids/sample. After collection, the petroleum ether was evaporated under a nitrogen flow in a laboratory hood until no petroleum ether was detected, and the vials were placed in a drying oven (100°C) for 30 min to evaporate any residual solvent, and transferred to a desiccator to cool for 30 min. The weight of the remaining lipids was used to calculate SLCs by expressing as a percentage of the original head rice sample (5 g). In order to determine significant differences among extraction temperatures, durations, and extraction systems for each rice variety, a Student's t-test $p<0.05$ using one-way analysis of variance (JMP IN 5.0., Cary, N.C.) was conducted.

RESULTS AND DISCUSSION

The extraction duration of 50-min generally produced SLCs greater than the 30-min extraction duration for 'Cypress' and 'Cocodrie' rice at all temperatures (Table 1). However, at the highest extraction temperature, 150°C, the Soxtec produced significantly lower SLCs for both 'Cypress' and 'Cocodrie' (0.46% and 0.43%, respectively) at the longest extraction duration of 50-min compared to the 30-min duration.

Comparing total extraction duration with the ASE system for 'Cocodrie' indicated that the total extraction length of 30 min (one 30-min cycle) generally resulted in similar or greater SLCs than the 50 min total extraction duration. This would imply that a complete surface-lipid extraction could be accomplished with the extraction duration of 30 min when using ASE. Comparing static cycles (one 30-min versus two 15-min cycles) on the ASE to determine if one cycle is as efficient as two consecutive cycles, results indicated that one 30-min cycle gave comparable or greater SLCs than two 15-min cycles (Table 2) except for 'Cocodrie' at 150°C. This would suggest that the total extraction duration might be more influential in increasing or decreasing the extraction efficiency than the number of static cycles. The extraction temperature of 150°C on the ASE produced significantly lower SLCs than the other extraction temperatures at all extraction durations, except for 'Cocodrie' at two 15-min cycles. These results concur with a study conducted on pressurized liquid extraction of medicinal plant extracts in which the extraction efficiency increased with increasing temperature up to a specific point, 120°C, and then declined when extracted at a higher temperature, 140°C (Ong et al., 2000). Generally, a higher temperature had more impact on lowering SLCs than the number of static cycles did when extracting surface lipids by ASE for both 'Cypress' and 'Cocodrie'. It could be reasoned then that one 30-min extraction cycle at temperatures 135°C or lower produces maximum SLCs when using the ASE system.

Table 3 shows the average SLCs of 'Cypress' and 'Cocodrie' compared between the two extraction systems for the 50-min extraction length, which is also illustrated more dramatically in Fig. 1, and then compared between the Soxtec at 50-min extraction and the ASE at 30 min (one 30-min cycle). The 50 min extraction duration was chosen since it was a better comparison between the two systems due to the 25-min boiling and 25-min rinsing with the Soxtec system that would be analogous to two 25-min extraction cycles when using the ASE system. Overall, it was observed that SLCs when extracted by ASE were equivalent to or greater than those obtained by the Soxtec system for both 'Cypress' and 'Cocodrie' (Fig. 1). This is similar to findings of other investigators who noted that when measuring polychlorinated biphenyl from various spiked organic matrices, the ASE had comparable to or slightly higher extraction efficiencies than those obtained by Soxhlet (Abraha and Raghavan, 2000). Wang et al. (1999) found polycyclic aromatic-hydrocarbon recoveries from several biological samples by the ASE method were comparable to or better than those obtained by Soxhlet extraction. In addition, using the ASE system reduced the extraction time by 20 min per sample since it produced SLCs with a 30-min extraction as great as the Soxtec system which produced acceptable SLCs with a 50-min extraction. Although 'Cocodrie' was not included in the 30-min ASE vs. the 50-min Soxtec comparison, the SLCs produced were similar or greater when using ASE at the 30-min extraction duration (Table 2) compared to SLCs produced using the Soxtec at the 50-min extraction duration (Table 1). Also notable was the reduction in solvent consumption between the two systems. For example, ASE used approximately 25 mL solvent/sample and the Soxtec system 70 mL/sample.

In our current study, overall the ASE provided surface-lipid determinations that were as reliable as are those obtained by the Soxtec system. This would suggest that the ASE is as thorough in extracting surface lipids as are commonly used conventional methods for DOM

determinations with the former offering advantages of shorter extractions, full automation, reduction in the amount of organic solvents required for extraction, and less handling required by the operator.

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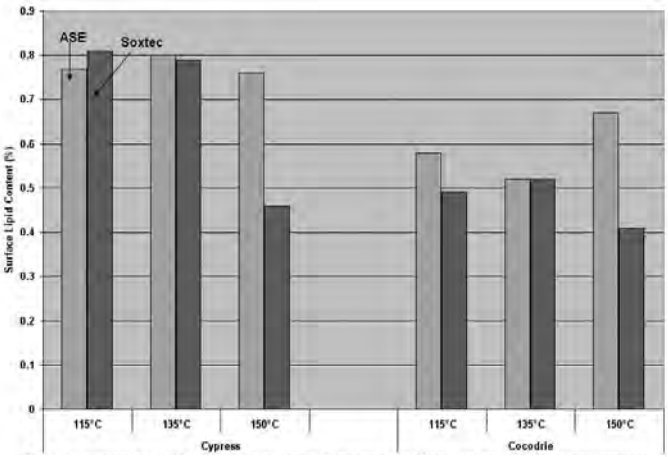

Fig. 1. Average surface-lipid contents of 'Cypress' and 'Cocodrie' rice at 50-min total extraction compared between ASE and Soxtec.

Table 1. Average surface-lipid contents (% of original head rice mass) of 'Cypress' and 'Cocodrie' rice using the Soxtec extraction system.

Temperature (°C)	Cypress		Cocodrie	
	Extraction durations			
	30 min[z]	50 min	30 min	50 min
115[y]	0.54Bb	0.81Aa	0.45Bb	0.49Ab
135	0.62Bab	0.76Aa	0.46Bb	0.52Aa
150	0.77Aa	0.46Bb	0.49Aa	0.43Bc

[z] Values between extraction durations within each temperature for each rice cultivar with different capital letters are significantly different ($p \leq 0.05$) by student t-test.

[y] Values within each rice cultivar for each extraction duration with different lowercase letters are significantly different ($p \leq 0.05$) by student's t-test.

Table 2. Average surface-lipid contents (% of original head rice mass) of 'Cypress' and 'Cocodrie' rice using accelerated solvent extraction.

Temperature (°C)	Cypress[z]			Cocodrie		
	Cycles[y]					
	1 30-min	2 15-min	2 25-min	1 30-min	2 15-min	2 25-min
115	0.70Aa	0.78Aa	0.80Aa	0.77Aa	0.63ABa	0.59Ba
135	0.81ABa	0.73Ba	0.84Aa	0.79Aa	0.61ABa	0.53Bb
150	0.45Bb	0.38Bb	0.76Ab	0.54Bb	0.61Aa	0.52Bb

[z] Values within each temperature for extraction cycles of each rice cultivar with different capital letters are significantly different ($p<0.05$) by students t-test.

[y] Values within each extraction cycle for each rice cultivar with different lowercase letters are significantly different ($p<0.05$) by student's t-test.

Response of blackberry cultivars to nematode transmission of Tobacco ringspot virus

Alisha Sanny[*]*, John R.Clark*[†]*, and Rose Gergerich*[§]

ABSTRACT

A study was conducted on eight cultivars of blackberry ('Apache', 'Arapaho', 'Chester', 'Chickasaw', 'Kiowa', 'Navaho', 'Shawnee', and 'Triple Crown'), of which four plants of each were previously determined in the fall of 2001 to have root, but not leaf, infection with Tobacco ringspot virus (TRSV). The objectives of our study were to determine virus effects on plant vigor and the spread of virus infection in the plants. Eight plants of each cultivar, four infected and four free of infection, were grown in pots on a gravel pad for the 2002 growing season, and samples of primocane and floricane leaves were taken to determine if TRSV had moved to the above-ground portion of the plants. TRSV infection was determined by ELISA tests. At the end of the growing season (October), the plants were harvested and dry weights determined for floricanes, primocanes, and roots to determine virus effects on plant vigor. In all plants that had been shown to have root TRSV infection, the virus was shown to have moved into the top portion of the plants as evidenced by positive ELISA tests on primocane and floricane leaf tissue. Dry-weight results indicated no significant interaction of virus infection and cultivar, or any main effects of virus on cane or root growth, as all dry weights were similar for infected and non-infected plants. No dramatic leaf symptoms of virus infection were observed on infected plants in our study at any time during the growing season. Further research should focus on possible virus effects on plants that have been infected for a longer period of time to determine if in fact the virus has any effect on plant growth or productivity.

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MEET THE STUDENT-AUTHOR

Alisha Sanny

In May 2003, I graduated *summa cum laude* with a degree in horticulture management and production. As an undergraduate, I was fortunate to have many splendid work and travel opportunities. I spent time working in the horticulture research plots at the Arkansas Agricultural Research and Extension Center, Fayetteville, the Horticulture Display Gardens on campus, and the Plant Pathology Virology lab. The work I did under Dr. John R. Clark at the horticulture farm led to my position as an Adair Intern in the Plant Pathology Department. The research I did during that internship is presented in this paper. As an undergraduate I was also actively involved in the Horticulture Club and the Razorbacks for Christ. In the Horticulture Club, I spent many hours preparing for plant sales, and I was able to travel to Florida for the American Society of Horticultural Science-Southern Region Annual Meeting. The Razorbacks for Christ provided a place to grow spiritually and cultivate leadership skills and friendships. Through the Razorbacks for Christ, I was able to go to Romania and Ukraine on mission trips. My last semester of college was spent at the Scottish Agricultural College in Ayr, Scotland.

I would like to thank everyone, especially Dr. John R. Clark, Dr. Rose Gergerich, and Scott Karnes, who contributed to my taking learning far outside the classroom, which greatly enriched my college experience.

INTRODUCTION

There have been 26 virus or virus-like diseases reported for *Rubus* crops (blackberries and raspberries) in the world (Jones, 1986). Viruses cause more damage in the black and purple raspberries and less damage in red raspberries and blackberries (Crandall, 1995). Many viruses that infect blackberry do not produce distinctive symptoms, and reports of virus effects on blackberries are very limited. A recent study on the impact of Raspberry bushy dwarf virus (RBDV) on 'Marion' blackberry in Oregon showed that there was no virus effect on cane number or length in a two-year period, but that there was a significant yield reduction (50%) in RBDV infected plants, along with reduced berry weight (40%) and drupelet number per berry (39%) (Strik and Martin, 2002). Infected plants also showed visual symptoms, including chlorosis, vein clearing, silver discoloration, and malformed small fruit. Newly infected plants did not display such distinct symptoms.

There are approximately 200-400 acres of blackberries grown and marketed locally throughout Arkansas. Eleven licensed Arkansas nurseries and 29 licensed nurseries in other states and countries propagate University of Arkansas patented cultivars, as well as other cultivars, for national and international markets (Troxell, 2001). The presence of virus symptoms in nurseries and commercial blackberry fields in Arkansas has been a recent cause for concern. A field survey was conducted in Arkansas of blackberry nurseries for TRSV, RBDV, and Impatiens necrotic spot virus (INSV) in 2002. All three viruses were found, but TRSV was found first and was most prevalent (Rose Gergerich, unpublished).

Leaves of blackberry and dewberry plants in North Carolina infected with TRSV showed faint to severe ringspots, mottling, mosaic, stunting, leaf distortion, and yellow line patterns (Rush and Gooding, 1970). However, they usually did not have symptoms on each cane. Virus symptoms on blackberry plants from Arkansas showed chlorosis, oak-leaf patterns, and mosaic (Troxell, 2001; Fig. 1).

Guzman et al. (2002) more recently conducted a virus survey in North Carolina, South Carolina, and Virginia in 2001-2002. In North Carolina, TRSV was found in

57% (257/451) of the symptomatic plants tested. TRSV was identified in 33% (21/62) of the samples from South Carolina, but TRSV was not identified in any of the plantings tested in Virginia. TRSV was detected in 'Apache' and 'Arapaho' most frequently (>50%). It was also detected in 'Chester', 'Chickasaw', 'Lochness', and 'Rosborough'.

TRSV is a nepovirus that was first reported in *Nicotiana tabacum* by Fromme et al. (1927). The first report of TRSV in blackberry was published in 1965 from North Carolina (Stace-Smith, 1987). Rush and Gooding (1970) isolated TRSV from *Rubus allegheniensis, R. argutus*, *R. flagellaris*, and an unidentified *Rubus* species in North Carolina. TRSV has a large host range, including both herbaceous and woody plants (Stace-Smith and Hansen, 1974). It occurs throughout North America, especially in the southeastern United States (Rush and Gooding, 1970).

TRSV virions are isometric, not enveloped, and 25-29 nm in diameter. The genome consists of two single strands of linear RNA, both of which are needed for infection (Brunt, et al., 1996). The primary spread of TRSV in the field is by the dagger nematode, *Xiphinema americanum*. The virus does not multiply in the vector and is lost once the nematode molts. It can also be transmitted mechanically, by infected nursery stock, and by pollen (Brunt, et al., 1996).

In the most extensive study of TRSV on blackberry, Troxell (2001) conducted nematode transmission experiments on eight blackberry cultivars (Apache, Arapaho, Chester, Chickasaw, Kiowa, Navaho, Shawnee, and Triple Crown). She infected tissue-cultured plants with TRSV using *X. americanum* transmission, and found all cultivars were susceptible to this virus as determined by sampling roots of exposed plants and testing for TRSV using Protein-A ELISA tests. ELISA tests revealed that TRSV was not present in the leaves of aboveground portions of these plants during the first growing season following nematode transmission of the virus. Symptoms were seen on primocane leaves of infected plants, but these were mild and transient.

Our study was initiated to further evaluate the virus effects on virus-infected or non-infected plants used in 2001 by Troxell (2001). Specifically, we wanted to determine the impact, if any, of TRSV infection in the second year following nematode transmission on blackberry plant vigor and to find if the virus could be found in aboveground plant portions based on ELISA tests.

MATERIALS AND METHODS

In March 2002, four non-infected and four infected plants of each cultivar used in an earlier study (Troxell, 2001) were chosen for this study. The plants had been grown in 3-L plastic pots the previous season, and had been mulched with sawdust mulch over the winter to protect them from winter injury. In March 2002, the plants were removed from the mulch and pruned, leaving two 0.8-m floricanes. Floricanes were staked using 1-m plastic stakes. The plants were moved to a gravel pad at the Arkansas Agricultural Research and Extension Center, Fayetteville, and four plants (replications) of each cultivar/virus infection status combination were arranged in a randomized block design. Each pot was set up in 12.2-m rows on 1.5-m squares (Fig. 2). Starting in May, pots were fertilized every two weeks with one tablespoon Osmocote (19-6-12) (until July) and irrigated overhead twice daily for one hour (until September). Plants were observed from May to November for virus symptoms.

In July 2002, Protein-A ELISA, as described by Edwards and Cooper (1985), was used for the detection of TRSV in the floricanes and primocanes of all plants. Leaf extracts were prepared by grinding floricane and primocane leaves with a sap extractor (Erich Pollahne Co., Wennigsen, West Germany). The sap was diluted 1:10 (v:v) in extraction buffer (10.3 mM Na_2SO_3, 0.5 mM polyvinylpyrrolidone M.W. 40,000 (PVP-40), 0.2% powdered egg albumin, 2% Tween-20 in PBS-T [137.8 mM NaCl, 1.47 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 2.68 mM KCl, 0.05% Tween-20; pH 7.4]). Immulon 1 flat-bottom microtiter plates (Dynex Technologies, Inc., Chantilly, Va) were coated with Protein-A (Sigma Chemical Co., St. Louis, Mo.) at 1µg/mL in coating buffer (15 mM Na_2CO_3, 34.88 mM Na_2CO_3, 3.08 mM NaN_3; pH 9.6). Plates were then coated with polyclonal antiserum to TRSV (from University of Arkansas plant virus antiserum collection) that had been diluted 1:1000 with PBS-T. After addition of the diluted leaf extract to duplicate wells of the plate, TRSV antiserum diluted 1:1000 in PBS-T was added to the plates followed by Protein-A alkaline phosphatase conjugate (Sigma) at 1µg/mL in PBS-T). Finally, nitrophenyl phosphate at 1 mg/mL (Sigma) in substrate buffer (0.39 mM $MgCl_2$, 3.84 mM NaN_3, 12.1% diethanolamine; pH 9.8) was added to the wells. Between all steps, plates were washed three times with PBS-T. All reagents were used at 100µL/well, and incubations were at 24°C for 2 h, except for the incubation after antigen addition, which was at 4°C overnight. Absorbance at 405 nm was determined with a 7520 Microplate Reader (Cambridge Technologies, Inc., Watertown, Mass.).

Plants from three replications were defoliated in November 2002 by hand, and the floricanes and primocanes cut at the crown level (soil surface) and dried separately in paper bags for 4 days at 65°C. After canes were

dry, they were weighed and discarded. Roots were washed, dried in the same manner, and weighed. The fourth replication was kept for further observation. Dry weight data were analyzed as a two-factor randomized complete block by JMP (JMP, version 4.0. SAS Institute, Inc. Cary, N.C., 1989-2000).

RESULTS AND DISCUSSION

All plants of all cultivars that had tested positive in ELISA tests in root samples taken in 2001 tested positive for TRSV in primocane and floricane leaves in July 2002. The ELISA tests also demonstrated that the non-infected control plants continued to be virus free. The finding that virus was present in the leaves indicates that the virus moved from the roots to the aboveground portion of the plants in the second year after nematode transmission. No virus symptoms were observed on the leaves during the study on infected or non-infected plants.

For the dry-weight data, the analysis of variance indicated no significant (P=0.05) interaction of infection status and cultivar for the variables measured. Additionally, the main effect of virus infection status was not different for any variables, indicating no virus effect on plant vigor. The dry-weight means were similar for floricanes, primocanes, and roots (Table 1). Although no significant differences were found between infected and non-infected blackberry plants in this study, it is possible the primocanes that are now virus-infected will show symptoms of virus infection when they develop as floricanes next year. Cultivars averaged over plant-infection status were significantly different for dry weight of primocanes, floricanes, and roots (data not shown). This finding was not important for this investigation since our effort was to identify virus effects, not cultivar vigor differences.

Table 1. Main effect means (dry weight in grams) for non-infected vs. infected blackberry cultivars.

Virus status	Primocanes	Floricanes	Roots
Infected	160.1	31.3	590.5
Non-infected	156.5	26.5	640.3
Significance[z]	0.08	0.75	0.30

[z]Significance- F-test, P=0.05.

The most noteworthy finding of our study was the second-year presence of TRSV in leaf samples. This has a number of implications for management of blackberry virus diseases. First, it is important for nursery growers and regulatory agencies because plants that become infected in the field by nematode transmission may be carrying virus in their roots (the portion of the plant often used for propagation) but testing negative for virus in their leaves in ELISA tests and appearing healthy based on the lack of leaf symptoms. Second, the dynamics of TRSV movement in the plant are clearer from our results. The delay in virus movement from the roots to the aboveground portion of the plant was longer than expected. This area needs further study to determine when virus symptoms commonly appear on aboveground portions of the plant after nematode transmission. The plants that were not destroyed for analysis in this research will be observed next year after another period of dormancy to determine if leaf symptoms develop on the floricanes which became infected with TRSV during the last year.

Field-grown, mature blackberry plants that express viral symptoms often grow well and bear abundant, good quality fruit. This has raised the question: What is the effect of viruses such as TRSV on blackberries? Our data on second-year plants indicate no virus effect on total plant growth. However, the virus may have greater effects the third year as it spreads further in the plant. Also, our plants did not bear fruit, thus we were unable to determine if TRSV-infected plants produced fruit that was malformed, crumbly, or otherwise possibly affected by virus infection. Additionally, if blackberry plants become infected with more than one virus, the combined effects of these viruses often produce severe disease symptoms.

The concerns of blackberry nursery stock producers are somewhat different from those of blackberry fruit producers. Many states and countries have regulatory agencies that restrict the movement of plants that are not certified as virus-tested. Blackberry plants expressing symptoms of virus infection would be denied entrance by such regulatory agencies. Based on the results of the research reported here, the absence of virus symptoms in blackberry leaves should not be used to determine whether blackberry plants are infected with TRSV.

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Fig. 1. Symptoms of TRSV infection in 'Arapaho' blackberry.

Fig. 2. Blackberry plants in field trials, mid-summer, at the Arkansas Agricultural Research and Extension Center, Fayetteville.

Effects of virus infection on release of volatile organic compounds from insect-damaged bean, *Phaseolus vulgaris*

Sarah E. Sossamon[*], *Britney K. Jackson*[†], *and B. Alison Drumwright*[§], *Kenneth L. Korth*[‡], *and Gisela F. Erf*[‡‡]

ABSTRACT

Insects can serve as important vectors of plant pathogens, especially viruses. Insect feeding on plants causes the systemic release of a wide range of plant volatile compounds that can serve as an indirect plant defense by attracting natural enemies of the herbivorous insect. Previous work suggests that the Mexican bean beetle (*Epilachna varivestis*) prefers to feed on plants infected by either of two viruses that it is known to transmit: Southern bean mosaic virus (SBMV) or Bean pod mottle virus (BPMV). A possible explanation for the preferred feeding on virus-infected tissues is that the beetles are attracted by volatile signals released from leaves. The purpose of this work was to determine whether volatile compounds from virus-infected plants are released differentially from those emitted by uninfected plants. To test the hypothesis, common bean plants (*Phaseolus vulgaris* cv. Black Valentine) were inoculated with either BPMV, SBMV, or a mixture of both viruses, and infected plants were compared to uninfected plants. An Ouchterlony assay was used with SBMV- and BPMV-specific antisera to confirm the presence of virus in inoculated plants. RNA blot analysis was performed on tissue from each plant and indicated that a well-characterized defense gene, encoding phenylalanine ammonia-lyase (PAL), was not induced in systemic tissue following virus infection. Plant volatiles were collected—and analyzed via gas chromatography (GC)—from plants that were either undamaged or beetle-damaged. In undamaged plants, there were no measurable differences in profiles or quantities of compounds released by uninfected and virus-infected plants. After Mexican bean beetles were allowed to feed on plants for 48 h, injured plants released several compounds that were not released from undamaged plants. Lower quantities of volatile compounds were released from virus-infected plants suggesting that enhanced release of plant-derived volatile organic compounds is not the cause for attraction of Mexican bean beetles to virus-infected plants.

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[†] Britney K. Jackson is a junior majoring in poultry science.

[§] B. Alison Drumwright is a senior majoring in poultry science.

[*†§] Students who conducted this interdisciplinary team research project during the Spring 2002 as part of the Rotations in Agricultural Laboratory Research course; Sarah E. Sossamon is the primary author.

[‡] Kenneth L. Korth, teacher and faculty mentor regarding molecular and biochemical analyses, is an assistant professor in the Department of Plant Pathology.

[‡‡] Gisela F. Erf, teacher and faculty mentor regarding the immunology aspects of this project, is an associate professor in the Department of Poultry Science .

MEET THE STUDENT-AUTHORS

Sarah Sossamon

I graduated from Ozark High School in 2001. I am a sophomore poultry science major with a pre-vet emphasis. I have received many scholarships including a University scholarship, the Arkansas Game and Fish Commission scholarship, an FFA foundation scholarship, a Dale Bumpers College scholarship, and a poultry science scholarship. I am involved with many clubs and organizations on campus including Chi Alpha—of which I am the president—Collegiate 4-H/FFA, Pre-Vet Club, Poultry Science Club, and the equine program. I am also a member of the National Society of Collegiate Scholars.

As a student in the Poultry Science Department I have had many academic and hands-on opportunities, including this one. I started my research of plant pathology through a lab rotations class. Thanks to the help of Dr. Korth and Dr. Erf I was able to carry the research out further. Although this research is not directly related to my field of study, it has taught me a great deal about conducting a specific science experiment and the techniques used in experimentation. The skills I learned from the research will help me as I progress in the science field and have already helped me in some of the classes I am taking now.

Once I complete my undergraduate program I plan on attending veterinarian school and then practicing both large- and small-animal medicine.

Britney K. Jackson

I am a junior in the Poultry Science Department at the University of Arkansas. I grew up in Fayetteville and graduated from Fayetteville High School in the year 2000. I am working with the USDA in Agricultural Research Services concentrating on microbiological research. In this field I have continued to apply the research skills I acquired through this project.

B. Alison Drumwright

I am a senior poultry science major in the Poultry Science Department of the University of Arkansas. My hometown is Germantown, Tenn., and after my bachelor's degree is completed, I am hoping to pursue a career in veterinary medicine. I received a SURF grant to continue in this area of research.

INTRODUCTION

Plants are continually threatened by a wide array of organisms including disease-causing microorganisms and herbivorous insects. In response to these attacks plants have developed a complex defense system in which specific mechanisms are triggered in response to specific pests (Karban and Baldwin, 1997). As part of a response to insect damage, plants release volatile compounds from leaves. These volatiles can serve as semiochemical attractants to natural enemies of the herbivore such as predatory arthropods (Dicke and Sabelis, 1988) and parasitoid wasps (Turlings et al., 1991), thereby serving an indirect defensive role (Kessler and Baldwin, 2001). The release of volatiles is systemic, occurring from both damaged and undamaged leaves of wounded plants (Turlings and Tumlinson, 1992). At least three chemical classes are represented in the profile of volatiles released following herbivory (Paré and Tumlinson, 1999). Green-leaf volatiles—namely C6 alcohols and aldehydes terpenes, and products of the shikimate pathway such as indole—are released following damage. The release of many of the terpenes and indole is often delayed by nearly a full day following initial damage, indicating that stimulation of plant biosynthetic pathways might be required.

Insects frequently serve as vectors of plant pathogens, especially viruses. By retaining viral particles in mouthparts and foregut, Mexican bean beetle (*Epilachna varivestis* Muls.) serves as an important vector of plant viruses that infect several species of beans. Viruses are spread when regurgitated on other plants during feeding. Two common viruses spread in this way, each with a narrow host range, are the Bean pod mottle virus (BPMV) and Southern bean mosaic virus (SBMV). Symptoms of BPMV include mild leaf mottling; BPMV has a single-stranded (ss) RNA genome and is a member of the Comoviridae. Bean leaves infected with SBMV have pale-yellow lesions and mosaic symptoms; SBMV also contains an ssRNA genome and is the type member of the genus Sobemovirus.

A previous study shows that Mexican bean beetles prefer feeding on virus-infected plants compared to feeding on non-infected plants, as measured by the amount of leaf material the beetles consumed (Musser, et al., 2003). We derived several hypotheses for the beetle preference for virus-infected tissue including the possibility that there are chemical antifeedants in uninfected plants. Another explanation is that volatile compounds released by virus-infected plants could be attracting the beetles. We attempted to determine whether common bean plants differentially release volatile compounds and so we collected and analyzed volatile organic compounds from beetle-damaged plants that were either virus-infected or uninfected.

MATERIALS AND METHODS

Plants and Insects. Common bean plants (*Phaseolus vulgaris* L. cv. Black Valentine) were used for this study. Seeds were planted in LC-1 Sunshine Mix soil in 4.5-inch pots. Plants were watered daily and maintained in the greenhouse at 24°C under a 16:8 h light:dark regimen. Virus inoculation was conducted when plants were 10 d old. One leaf per plant was mechanically inoculated with either BPMV, SBMV, or a mixture of the viruses. Virus-containing plant sap was obtained from other previously infected bean leaves. Virus sap was prepared by grinding three to four infected leaves in a sterile mortar with 2 mL of ice-cold extraction buffer (50 mM potassium phosphate, pH 7.2). Leaf tissue was ground until no large pieces of tissue remained. The virus-containing sap was applied with a saturated cotton inoculation pad to a leaf that was lightly dusted with abrasive 600-mesh carborundum. Plants that were mock-inoculated with sap collected in the same manner from healthy plants served as controls throughout all experiments.

Mexican bean beetles and virus-infected leaves were graciously provided by Dr. Rose Gergerich and Sandy Wickizer, Department of Plant Pathology, University of Arkansas. Plants were exposed to 10 d after virus inoculation. A nylon net was placed over each pot containing two plants per pot to contain four beetles that were added and allowed to feed for 48 h.

Virus Identification and Detection

A standard Ouchterlony gel double-diffusion test in 1% agarose was used to identify and confirm viral infection of plants. Sap was extracted from virus-infected leaves by squeezing with a tissue-extractor. For this immunoassay, 35 µL of each virus extract was placed into labeled wells in the Ouchterlony gel plates. BPMV- and SBMV-specific antisera, diluted 1:10 in 50% glycerol, were placed into the center wells of duplicate Ouchterlony plates. Extracts from the mock-inoculated uninfected plants were used as negative controls for each test.

RNA Analysis

Bean plant RNA was isolated using TriReagent (Molecular Research, Inc., Cincinnati, Ohio) according to the manufacturer's instructions. Two leaves from each type of virus-infected plant were stored in liquid nitrogen until RNA isolation. For RNA isolation, leaves were ground to a fine powder using a sterile pestle and mortar containing liquid nitrogen. Final concentrations of RNA samples were determined spectrophotometrically by absorbance at 260 nm.

Total RNA was separated by electrophoresis in a 1% agarose formaldehyde gel and transferred to a nylon membrane via capillary transfer (Sambrook, et al., 1989). Membranes containing RNA were hybridized overnight (Church and Gilbert, 1984) with radiolabeled probes produced with ^{32}P in random-primer reactions (Sambrook, et al., 1989).

Analysis of Plant Volatile Compounds

Volatile compounds released from intact plants were collected by placing plants in 18-L glass chambers. Activated-carbon-filtered air was introduced at the top of each glass chamber at a rate of 1L/min. Gaseous contents of the chamber were removed by vacuum flow 0.8 L/min through a volatile-trap tube containing 75 mg SuperQ resin (Alltech, Inc., Deerfield, IL) near the bottom of each chamber. Excess introduced air was allowed to exit through the open bottom of each chamber. Volatile organic compounds bound to the resin were removed by washing with 250 μL of CH_2Cl_2 directly into glass gas-chromatography (GC) vials. GC was performed on a Hewlett Packard 5890 gas chromatograph equipped with Restek Rtx-1 column (30 m x 0.25 mm I.D.) essentially as described by Turlings, et al. (1991). Splitless injection of 2 μL per sample was performed and separation of compounds was monitored with a flame-ionization detector.

RESULTS AND DISCUSSION

Immunoassays Confirm Virus Infection

An Ouchterlony assay is a simple method used to confirm presence of a specific compound through a binding interaction between specific antibodies and a corresponding antigen. Briefly, antiserum and test material are placed separately into an agar-gel matrix and allowed to diffuse through the medium until their paths cross. If antibodies in the serum bind to an antigen, a band of visible precipitant forms in the agar. Location of the band of precipitation can vary depending on the relative concentration of antibody and/or antigen in solution. Ouchterlony assays confirmed that BPMV and SBMV were present and replicating in the individually inoculated plants (Fig. 1). In addition, both viruses were able to replicate in a mixed-inoculation, based on precipitation patterns that appeared in the gel. The absence of a precipitation signal in the mock-inoculated sample confirmed that there was no cross-contamination between study plants. Therefore, uninfected healthy plants did not contain BPMV or SBMV and virus inoculations were successful.

Defense Gene Induction in Response to Virus Infection and Beetle Herbivory

Phenylalanine ammonia lyase (PAL) is a critical enzyme in the production of defense-related phenolic compounds in plants (Bate, et al., 1994). The accumulation of the PAL enzyme is known to be controlled at the level of RNA transcription that increases depending on the developmental stage of the plant (Liang, et al., 1989) and in response to fungal pathogens (Cuypers, et al., 1988). Accumulation of PAL transcripts was compared among mature leaves of uninfected healthy plants and those infected with BPMV, SBMV, or a mixture of both viruses. Virus-infected plants contained PAL transcript levels similar to those in uninfected plants (Fig. 2).

Essentially identical results were obtained when this experiment was repeated (data not shown). The study was complicated by a low level of unwanted insects, namely thrips, in the greenhouse, which could account for some of the PAL gene expression in uninfected plants. Nonetheless, it seems clear that expression of the gene encoding this enzyme is not strongly induced using the viral treatments described here. The RNA blots were also probed with a radiolabeled 18S rRNA to confirm equal loading of total RNA on the gel (Fig. 2). Although many studies have been performed showing increased PAL gene expression in response to bacterial or fungal pathogens, there are surprisingly few studies measuring PAL expression in response to viral infections in intact common bean plants.

Volatile Compound Measurements

Herbivore-damaged plants emit volatile organic compounds as part of a defense response. The emitted compounds can serve as an indirect defense against insect pests by attracting natural enemies of the herbivore or alternatively they might act as a repellant to herbivores (Kessler and Baldwin, 2001). Thus, possible explanations for beetle preference for infected plant material could be that virus-induced plant volatiles attract beetles, or that uninfected leaves emit deterrents to beetle feeding. To test these possibilities, we allowed Mexican bean beetles to damage leaves (Fig. 3A) and then analyzed volatile organic compounds released from leaves into a glass chamber (Fig. 3B).

Virus infection had little effect on the profile of volatiles collected from undamaged plants, as compared to uninfected, undamaged plants (Fig. 4). This suggests that volatile release prior to plant damage is not the primary reason for beetle feeding preferences.

The number and amounts of volatile compounds released from undamaged plants was much lower than was observed in insect-damaged plants. As expected,

beetle feeding resulted in more volatiles being released when compared to undamaged plants (Fig. 5A). Although there have been few reports of analysis of volatiles released from common bean leaves, the effects of mite damage on volatile release from the closely related lima bean, P. lunatus, have been studied in detail. Lima bean, along with virtually every other plant species examined to date, releases 11-carbon (4,8-dimethyl-1,3,7-nonatriene; DMNT) and 16-carbon (4,8,12-trimethyl-1,3,7,11-tridecatetraene; TMT) homoterpenes in response to arthropod herbivory (Boland, et al., 1992). In addition, lima bean is known to release other terpene compounds along with green-leaf volatiles and products of the shikimate pathway (Dicke, et al., 1990; 1999). We observed that the amounts of herbivory-induced volatiles released from bean leaves that were virus-infected were much lower than in uninfected leaves (Fig. 5). Collection of volatiles was carried out three times in independent experiments and the quantification of selected peaks is presented in Table 1. The results illustrate the general trend of reduced volatile release from virus-infected leaves and also the occasional variation observed in levels of individual compounds analyzed in these experiments. The volatile collection apparatus consists of four identical chambers used in parallel with forced air and vacuum lines that are equally distributed to each chamber. Control tests verified that the variation is due to natural plant differences and not to the experimental apparatus or the GC analysis.

We consistently observed that a mixed viral infection, which gave the most severe plant symptoms of any of the treatments, resulted in the greatest reduction of insect-induced volatiles. We did not observe unique GC peaks following any of the treatments. The putative identifications of compounds quantified in Table 1 are based on identical retention times of the unknown peaks with known standard compounds. In addition, the release of the homoterpenes DMNT and TMT, and the terpenes β-caryophyllene and α-bergamotene, is well documented in a lima bean-mite system (Takabayashi, et al., 1991). Absolute confirmation of the identification of the compounds would require an analysis such as mass spectrometry.

Because uninfected bean plants released higher levels of volatile organic compounds, this might suggest that Mexican bean beetles prefer to feed on virus-infected plants because some volatiles act as insect repellants. However, prior to insect damage we observed no difference in volatiles released by uninfected or virus-infected plants, so any repellant activity must be occurring well after the insects begin feeding.

A more plausible explanation might be that enhanced volatile release is an indicator that uninfected plants are better able to mount a defense response than are virus-infected plants with compromised defense systems. This reasoning would account for the insect feeding preference for pathogen-infected plants. The pathogen-induced defense signaling pathways of plants can be distinct from those induced by chewing insects. Although results vary with the experimental system, a growing body of literature suggests that antagonistic interactions exist between pathogen- and insect-defense pathways in plants (Felton and Korth, 2000; Kunkel and Brooks, 2002). Because the Mexican bean beetle serves as a vector of both BPMV and SBMV, enhanced feeding on infected plants would seem to increase the probability

Table 1. Relative levels of insect-induced plant terpene volatiles released from virus-infected plants.

Retention time[w]	Putative I.D.[x]	Treatment[y]	Relative level +/- SD[z]
13.82 min	DMNT	BPMV	0.439 +/- 0.087
		SBMV	0.992 +/- 0.519
		Mix	0.599 +/- 0.054
22.08 min	β-caryophyllene	BPMV	0.346 +/- 0.320
		SBMV	0.712 +/- 0.534
		Mix	0.086 +/- 0.010
22.88 min	α-bergamotene	BPMV	0.329 +/- 0.288
		SBMV	0.672 +/- 0.496
		Mix	0.167 +/- 0.138
25.69 min	TMT	BPMV	0.472 +/- 0.264
		SBMV	0.779 +/- 0.570
		Mix	0.291 +/- 0.049

[w] Retention time of compound as determined by gas chromatography described in Materials and Methods section.

[x] Putative identification of terpenes is based on identical retention time of known standard compounds. DMNT, 4,8-dimethyl-1,3,7-nonatriene. TMT, 4,8,12-trimethyl-1,3,7,11-tridecatetraene.

[y] Volatiles were analyzed after collection from beetle-damaged plants that were infected with bean pod mottle virus (BPMV), Southern bean mosaic virus (SBMV), or both viruses (Mix).

[z] Numbers represent average relative amount of compound as based on GC peak areas, compared to amount of compound released from an uninfected insect-damaged bean plant analyzed in parallel. Results are compiled from three independent replicated treatments, n=3. SD = standard deviation.

that viruses would be taken up and spread to other plants (Musser, et al., 2003). Likewise, if plant defenses against insects are weakened by virus infection then the fitness of beetle populations might increase as a result of feeding on these plants. Therefore, both the pathogen and its vector could be mutually aided in this interaction. Further studies are necessary to determine if these hypotheses hold true.

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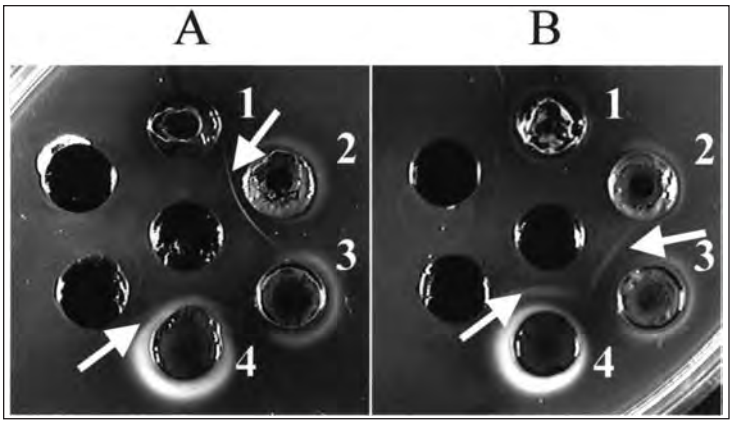

Fig. 1. Agar plates of Ouchterlony assays showing virus-antibody interactions. SBMV-specific (A) and BPMV-specific (B) antisera were added to the center well of each plate. Bean-leaf extracts added to the outside wells were 1) Uninoculated, 2) SBMV, 3) BPMV, and 4) Mixed infection. Arrow indicates antigen-antibody reaction near the well.

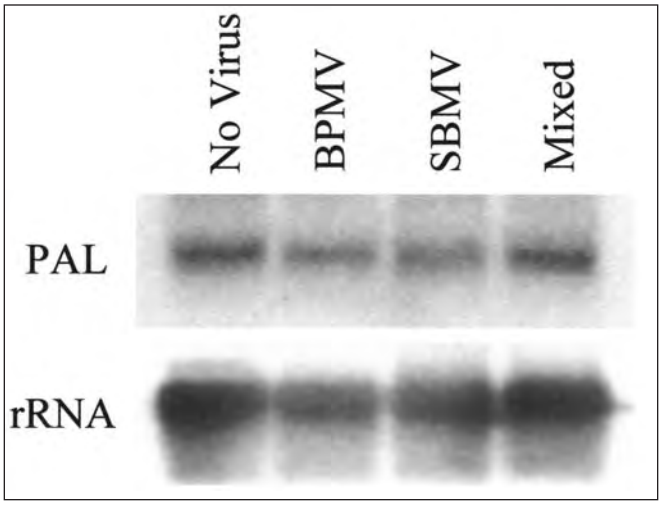

Fig. 2. Viral infection does not induce accumulation of transcripts for a defense gene. Autoradiographs showing presence and levels of mRNA encoding phenylalanine ammonia lyase (PAL) following gel electrophoresis and hybridization with a radiolabeled probe.

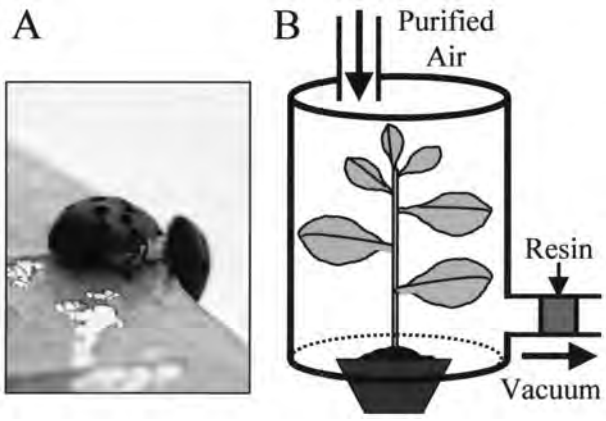

Fig. 3. A, Mexican bean beetle, *E. varivestis*, shown with feeding damage on a common bean, *P. vulgaris*, leaf. B. Schematic illustration of plant-volatile collection system described in the Materials and Methods section.

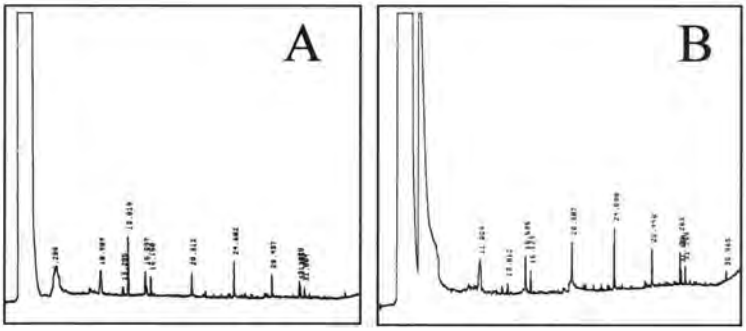

Fig. 4. Viral infection does not affect volatile release from undamaged plants. Gas chromatograph profiles are shown of separated compounds from undamaged bean plants that were either uninfected (A) or infected with a mixture of BPMV and SBMV (B).

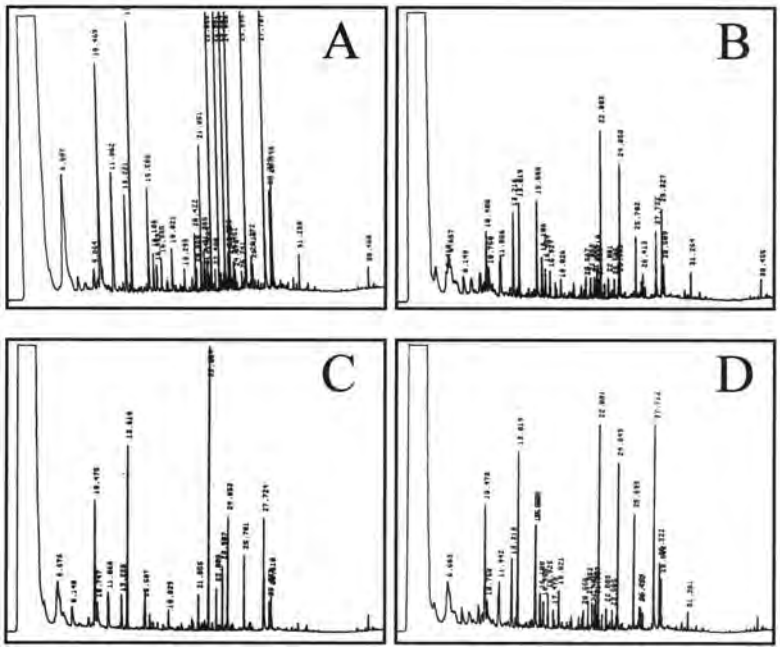

Fig. 5. Viral infection reduces the amount of volatile organic compounds released from bean leaves following beetle herbivory. Gas chromatograph profiles are shown of compounds from damaged bean plants that were either uninfected (A), infected with BPMV (B), SBMV (C), or a mixture of both viruses (D).

DNA sequence of melanocortin 1-receptor gene in *Coturnix japonica*: correlation with three *E* locus alleles, E, e^{+}, and e^{rh}

Rupali B. Ugrankar[*], *Kim Cheng*[†], *and Ronald Okimoto*[§]

ABSTRACT

The melanocortin 1-receptor (MC1-R) gene plays a key role in the expression of fur and feather color in mammals and birds by regulating the distribution of two melanin pigments: eumelanin (black/brown) and pheomelanin (red/yellow). MC1-R corresponds to the classical *Extension (E)* locus in mice, pigs, dogs, horses, and chickens. Three *E* locus alleles, the wild-type (e^{+}), brown (E), and redhead (e^{rh}) have been identified in Japanese quail (*Coturnix japonica*). To determine if the quail *E* locus phenotypes were due to variation in the MC1-R gene, the coding region of the MC1-R gene was PCR amplified and DNA sequenced using genomic DNA isolated from individuals exhibiting the phenotypes of the three quail alleles. The DNA sequence comparison revealed two missense mutations that differentiated the brown from the wild-type and redhead quail. A single-base substitution resulted in a Val58Ile change, and another single-base substitution produced a Glu92Lys change in the brown quail. The redhead quail sequence carried a seven-base deletion extending from nucleotide position 682 to 688, resulting in a reading frame shift and premature termination of the MC1-R gene after amino acid position 231. The Glu92Lys change in the brown allele created a *Msc* I restriction fragment length polymorphism (RFLP). A PCR-*Msc* I RFLP test was developed and a direct correspondence between phenotype and genotype was found by testing the DNA of a population segregating for the brown and wild-type alleles. The DNA sequence and segregation data indicate that the quail *E* locus is homologous to the *E* locus identified in other birds and mammals.

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MEET THE STUDENT-AUTHOR

Rupali B. Ugrankar

I am an international student from Bombay, India, where I completed my high school education at Jai Hind College. In May 2003 I completed my B.S. degree in environmental, soil, and water sciences with honors, graduating Summa Cum Laude and Senior Scholar. I was named Presidential Scholar for 2002-2003 and have received numerous other scholarships including International Student Scholarship, C. Roy Adair Scholarship, Fontaine Earle Crop Science Scholarship, RP and Mildred Bartholomew Memorial Scholarship, and Gamma Sigma Delta Scholarship. I was a Bumpers College Ambassador during 2001-2002 and am a member of Gamma Sigma Delta agricultural honors society and Golden Key Honors Society.

In fulfillment of my Honors Program requirements, I selected a genetics project, completely unrelated to my major. During the course of my research I learned valuable molecular techniques, and my interest in genetics grew. I will be pursuing this interest further when I embark on a Ph.D. degree program in molecular genetics and microbiology at the University of Texas at Austin, in Fall 2003.

INTRODUCTION

The melanocortin 1-receptor or MC1-R gene corresponds to the *E* or *Extension* locus in mice, pigs, dogs, horses, sheep, fox, cows, and chickens (Kijas et al., 1998; Lu et al., 1996; Takeuchi et al., 1996). Polymorphisms in the MC1-R gene affect the distribution of the two melanin pigments- black/brown eumelanin and red/yellow pheomelanin in the fur of these mammals and in the feathers of chickens. Dominant mutations at the *E* locus result in a constitutively active MC1-R generating a uniformly black coat or feather color, while recessive mutations at this locus usually eliminate receptor activity, producing more red and yellow pigmentation (Kijas et al., 1998).

Relative to the above animals, few feather pigmentation studies have been conducted on the Japanese quail (*Coturnix japonica*). In fact it is not known if the *E* locus in Japanese quail is homologous to what is called the *E* locus in chickens. But considering that the Japanese quail and chickens are close relatives, it is expected that the same genetic mechanisms, with regard to feather color, may apply to both species (Cheng and Kimura, 1990). Three quail *E* locus alleles, the wild-type (e^{+}), brown *(E)*, and redhead (e^{rh}) have been identified by the efforts of Truax (1979), Truax et al. (1979), and Truax and Siegel (1981), but specific polymorphisms giving rise to these phenotypes have not been studied. Fig. 1 shows the phenotypes of the brown, wild-type, redhead, and shafted quail (E/e^{+}).

There are several reasons why the Japanese quail has recently become a subject of biological research. Domesticated in the 11th century, it was used as a songbird and later as a source of meat and eggs. Amateur bird hobbyists and fanciers find them easy to raise because they are hardy and easy to manage. Due to the Japanese quail's short reproductive cycle of only 35 days, it is ideal as a model species for comparative studies (Ernst, 2000). In spite of its small body size and cryptic coloration, it is widely consumed in the Middle East, Asia, and Europe, but its consumption in North America and Australia has remained low as it has been considered more of a specialty item (Cheng and Kimura, 1990). However, recently its popularity as a delicious food source has increased, and many people enjoy pickled hard-boiled quail eggs (Ernst, 2000). Considering that feather pigmentation potentially affects tissue/meat coloration and, therefore, affects the consumers' perceptions of quail meat as an appealing food source, it is important to understand the molecular genetics of melanin production in quail feathers.

Through this study we sought to identify the polymorphisms in the *E* locus that are associated with the different feather-color phenotypes in Japanese quail.

MATERIALS AND METHODS

Polymerase chain reaction and DNA sequencing

Blood was extracted from the wing veins of six Japanese quail: two brown (*E/E*), two wild-type (e^+/e^+), and two shafted (E/e^+) birds. Blood samples of two redhead (e^{rh}/e^{rh}) were shipped from Canada. DNA was isolated from the whole blood using a Qiagen Blood Minikit (Qiagen, Valencia, CA), quantitated, and diluted to 20 ng/µl. We initially employed chicken MC1-R primers for polymerase chain reaction (PCR) amplification using Promega (Promega, Madison, WI) or Biolase (Bioline, Canton, MA) Taq polymerase. Thermocycler conditions for PCR were 94°C 30 s denaturation, 55°C 1 min annealing, and 72°C 1 min elongation for 35 cycles. A 0.8 or 1.0% TAE agarose gel electrophoresis of the PCR product separated the DNA bands, which were extracted using dialysis-filter paper method of Girvitz et al. (1980). The DNA isolated was sequenced using Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) on the ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). The oligonucleotide primer sequences used in this study are listed in Table 1. Chicken MC1-R primers +MC977 and –MC1132 amplified the 3' end of only wild-type MC1-R. From this initial sequence data, we designed the –QMC1-1522 quail MC1-R primer that was coupled with +MC977 to amplify the 3' end of the brown sequence. Using chicken and the new-quail MC1-R sequences, we designed several specific quail MC1-R primers for the 5' and 3' ends of the coding region of the MC1-R gene. Other notable primers used were +MC1R-340 and –QMC1087 for wild-type sequencing and +QMC-77 and –QMC1087 for brown sequencing. Relative to the wild-type, we faced considerable difficulty in amplifying the brown MC1-R coding region. On one occasion, we had to resort to a nested PCR technique to obtain the brown sequence (i.e. we re-amplified the DNA isolated from the agarose gel using the same primers +QMC-102 and –QMC1087, re-isolated the DNA from the agarose gel, and then sequenced the DNA using primers +QMC-77 and –QMC1087). The same steps as above were repeated for the redhead. Notable redhead MC1-R primers were +MC1R600 and –QMC-1535, and +MC1R-340 and –MCR11005. All DNA sequencing was done at the DNA Resource Center of the University of Arkansas, Fayetteville. The brown, wild-type, and redhead MC1-R sequences were assembled and analyzed using the DNAstar software package (DNAstar, Madison, WI).

PCR-RFLP segregation study

Four quail matings were set up in separate cages. Two cages housed one wild-type male with two shafted females, and the other two had one shafted male with two wild-type females. Eggs were collected for 2 weeks before they were incubated in petersime incubators. One of the wild-type male-shafted female mating was infertile and did not yield any chicks. A second round of matings was set up for the three that worked. Chicks obtained from both matings were typed as wild-type or shafted at time of hatch and typed again as adults. Ratio of wild-type progeny to shafted progeny was determined. Blood samples were taken from the parents and the progeny, and DNA was isolated from whole blood using Qiagen blood minikit. A PCR-RFLP (RFLP = Restriction Fragment Length Polymorphism) test was performed on the DNA from all the wild-type and shafted parents and progeny. A segment of the MC1-R was amplified by PCR using primers +QMC1Rq401 and –MCR11005. The PCR product was treated with restriction enzymes *Msc* I at 37°C for one hour, and then run on 1.5% TAE ethidium bromide-stained (0.15mg/ml) agarose gel. The arrangement/separation of the DNA bands on the gel for the wild-type and shafted progeny was examined under ultra-violet light, and a picture of the ethidium bromide stained gel was taken using the fluorescence setting on the Fujifilm Intelligent Dark Box II (Fujifilm, Edison, NJ). The entire MC1-R coding sequences of the brown, wild-type, and redhead quail were assembled using DNAstar software package

RESULTS AND DISCUSSION

Examination of the sequence data of the brown, wild-type, and redhead quail revealed some notable differences between the three. The wild-type and redhead quail had amino acid valine at position 58, while the brown quail had isoleucine at the same position owing to a single-base substitution of adenine (A) for guanine (G) at nucleotide position 172. In addition, the wild-type and redhead quail had glutamic acid at position 92, while the brown quail had lysine resulting from a single-base substitution of A for G at nucleotide position 274. Interestingly, a similar Glu92Lys substitution is found in chickens and somber-3J black mice. Apparently this single-nucleotide polymorphism results in a constitutively active MC1-R in both species and produces a solid black phenotype in these species (Takeuchi et al., 1996). In quail this mutation is associated with the extended-brown phenotype. In chickens, the *E* allele referred to as extended black is almost completely dominant over all other *E* locus chicken alleles, viz., birchen (E^R); dominant wheaten (e^{Wh}); wild-type (e^+); brown (e^b); speckled (e^s); buttercup (e^{bc}); and recessive wheaten (e^y) (Smyth, 1990). Similarly, the *E* allele in Japanese quail, conferring the brown phenotype, is incompletely dominant over the wild-type e^+ allele and completely dominant

over the redhead e^{rh} (Truax and Siegel, 1981).

Other than these missense single-nucleotide polymorphisms, three silent single-base substitutions were found. At nucleotide position 177, the wild-type and brown had thymine (T), while the redhead had cytosine (C). The brown and redhead had T at nucleotide position 513, and the wild-type at C instead. Finally, at nucleotide position 627 the brown and redhead had C, while the wild-type had T.

We discovered a significant mutation in the redhead sequence. A seven-base deletion extending from nucleotide position 682 to 688 resulted in the shifting of the reading frame and premature termination of the MC1-R gene after amino acid 231 (Fig. 2). This is expected to reduce the activity of the MC1-R because the shorter gene will fail to produce a full-length polypeptide and may be responsible for the dilution of color in the redhead plumage. In cattle a frameshift mutation in the dominant allele *ED* causes premature truncation of the receptor and produces red coat color (Klungland et al., 1995).

Truax (1979) crossed the brown and wild-type quail, which yielded all shafted progeny in the F1 generation. He concluded that the brown *E* allele was incompletely dominant over the wild-type (e^+) allele. We set up four test crosses between wild-type (e^+/e^+) and shafted (E/e^+) parents, as described in the materials and methods section. According to Mendelian ratios, we expected that the progeny would segregate 1:1 for the wild-type and shafted. Our results were skewed, but the difference was not significant by chi-square analysis ($\chi^2 = 2.8$): of the total 70 progeny obtained, 28 were wild-type and 42 were shafted, giving instead a wild-type:shafted ratio of 1:1.5.

Analysis of the brown, wild-type, and redhead MC1-R sequences revealed that the A to G substitution at nucleotide position 274 responsible for the Glu92Lys change in the brown allele creates a palindromic sequence TGGCCA, recognized by the restriction enzyme *Msc* I. The *Msc* I enzyme was expected to cleave the brown allele, but not the wild-type or redhead allele. The +QMC1Rq401 and -MC1R1005 primers were used to amplify a 433 base-pair (bp) segment of the MC1-R gene of the test cross parents and the 70 progeny. The *Msc* I was expected to cleave the 433 bp fragment twice. The brown DNA fragment would be cut into 56 bp, 158 bp and 219 bp fragments. The wild-type allele is cleaved once and produces a 377 bp and 56 bp fragment. The shafted quail carry one wild-type allele and one brown allele, hence three bands were observed for the shafted parents and progeny: an uncut wild-type band at 377 bp, and the additional two bands at 158 bp and 219 bp. The 56 bp fragment was difficult to observe on the gel (Fig. 3). The PCR-RFLP test confirmed that there was a 1:1 correspondence of the Lys92 mutation with the dominant brown phenotype, indicating that this mutation in the MC1-R gene was associated with the *E* locus phenotype.

ACKNOWLEDGMENTS

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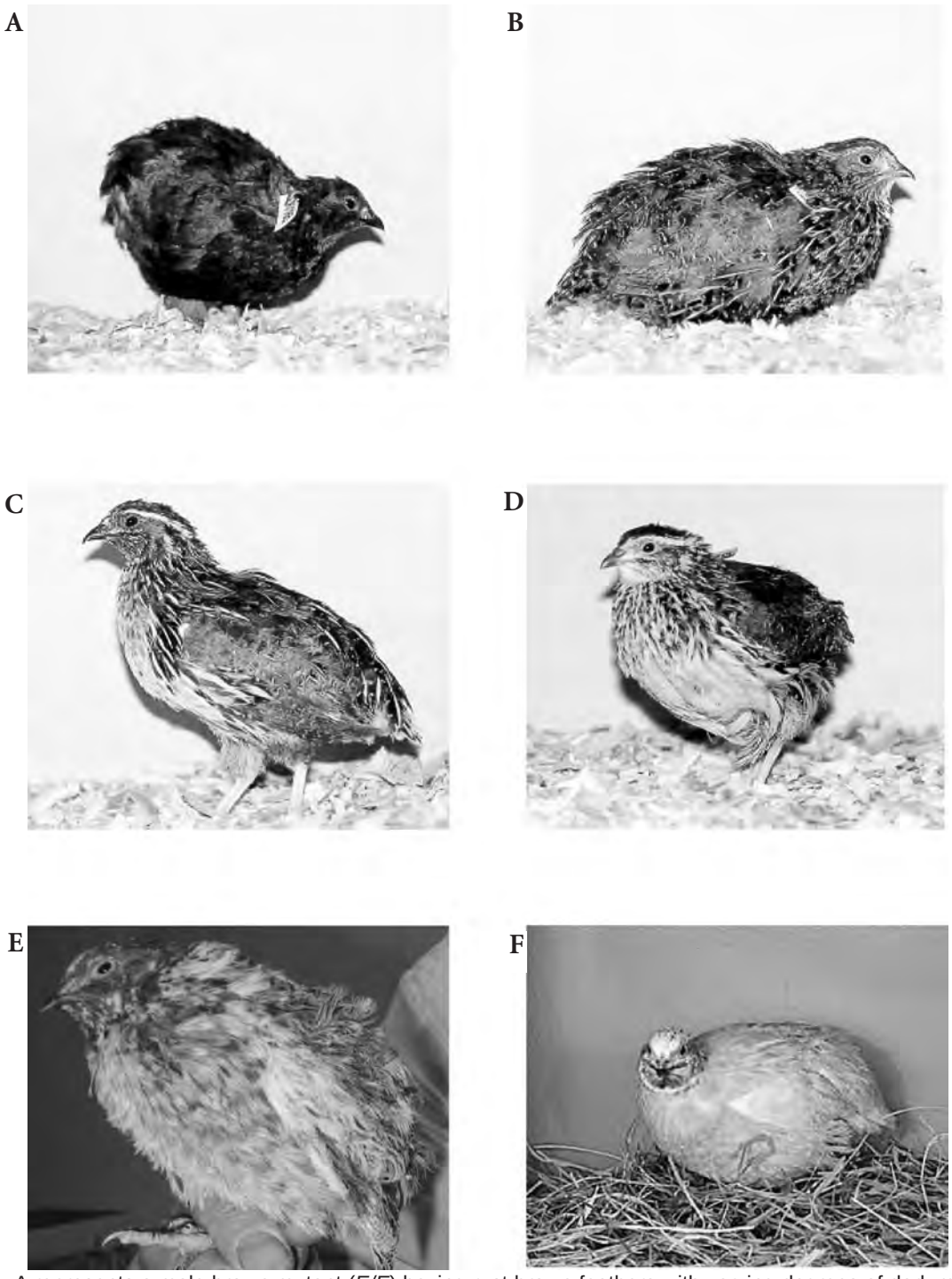

Fig. 1. A represents a male brown mutant (*E/E*) having rust brown feathers with varying degrees of dark markings along with dark brown legs, toes, and beaks, but the bottoms of the feet are light (Truax and Siegel, 1981). Fig. 1B is a female shafted quail (E/e^{+}) with brown feathers similar to the brown mutant in addition to light yellow shafting on the body plumage (Truax, 1979). Fig. 1C and 1D represent a male and female adult wild-type (e^{+}/e^{+}), respectively. Their plumage is a mix of several colors, but black and numerous shades of brown predominate on the dorsal side. Brown plumage is crossed by a black bar, and wheat-straw colored shafting occurs on the back and hackle feathers (Somes, 1979). Fig. 1E and 1F represent male and female redhead mutants (e^{rh}/e^{rh}), respectively. Phenotype is predominantly white with irregular black and rust colored markings, and beak color may range from a mixture of pink and black to solid black; the toes and legs are pink with black markings (Truax and Siegel, 1981).

Table 1. Oligonucleotide primer sequences used in this study

Primer name	Sequence (5'-3')
+MC977	CAGCACCGTCTTAATCACCTACTA
+MC-117	TGCTGCGGGAGCACTGGT
+MC805	ATGTCATCGACATGCTCATCTG
+MC1R-77	GGCTTTGTAGGTGCTGCAGTTG
+MC1R-400	GGGCACAGGCTGTCATGTG
+MC1R-340	GCCAGCTTTAAATCAGGACAGAG
+MC1R600	CTGGTGAGCCTGGTGGAGAAC
+MC1R-153	GCAGAGGTGCCCACATCC
+QMC1-71	GTAGGTGCTGTGGTTGTGCTC
+QMC1-495	CCCTGGAACGCCACTGAG
+QMC-102	GCTGGCAGGGCTGATAGG
+QMC-77	GGGGCTTTGTAGGTGCTG
+QMC1Rq401	CCCCAATGAGCTGTTCCTGAC
-MC1132	AGCCTTTATTTGGGAGCGCGA
-MCR11005	GCGGTAGTAGGTGATTAAGAC
-QMC1087	AACATGTGGATGTAGAGCACCAG
-QMC1135	CTGCTTCTGCTGGCTGGAGAT
-QMC1-1535	CCCACAGTGCGTCCGTC
-QMC1-1089	GCGCAAACATGTGGATGTAG
-QMC1-1522	CACAGTGCGTCCGTCTGTC

Allele	Amino Acid Positions									
e^+	225	226	227	228	229	230	231	232	233	234
	CAG	CCC	ACC	ATC	TAC	CGC	ACC	AGC	AGC	CTG
	Gln	Pro	Thr	Ile	Tyr	Arg	Thr	Ser	Ser	Arg
e^{rh}	225	226	227			228	229	230	231	232
	CAG	CCC	ACC	---	---	-GCA	CCA	GCA	GCC	TGA
	Gln	Pro	Thr			Ala	Pro	Ala	Ala	TER

Fig. 2. Comparison of the DNA sequence of the wild-type (e^+) and the redhead alleles (e^{rh}), where a seven-base deletion in the redhead sequence produces a stop codon at amino acid position 232, causing premature truncation of the coding region of the MC1-R gene.

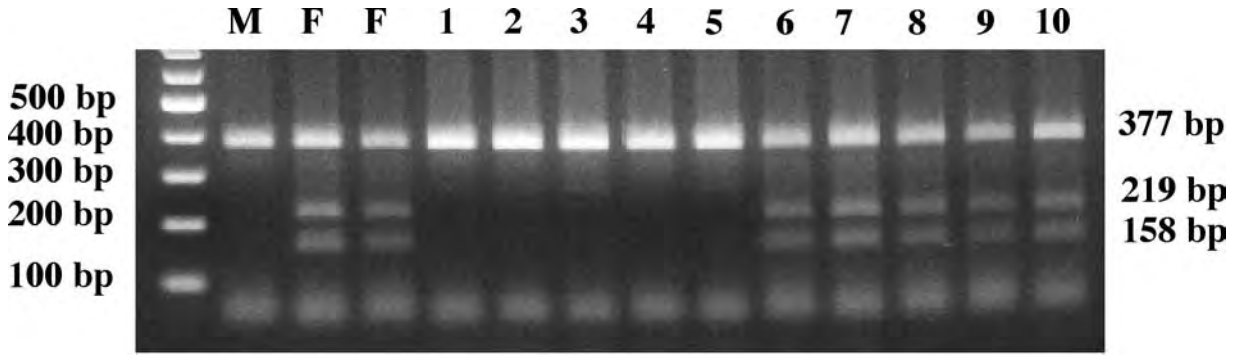

Fig. 3. The ethidium bromide stained 1.5% agarose gel shows the arrangement of *Msc* I digested MC1-R DNA fragments of one wild-type male (M) and two shafted female (F) parents and their five wild-type (1 to 5) and five shafted (6 to 10) progeny.

Soil particle-size analysis: A comparison of two methods

Lauren A. Williams-Caudle[*]*, Kristofor R. Brye*[†]*, and E. Moye Rutledge*[§]

ABSTRACT

Knowing the proportion of particle sizes in soil is important to soil scientists and agronomists. The mixture of sand, silt, and clay influences water movement, solute transport, nutrient retention, and many other properties and processes in soil. The standard method for particle size determination is a somewhat time-consuming process. An equally accurate but shorter method would be appealing for many reasons. The objective of this study was to compare a standard method of particle-size analysis using a hydrometer to an abbreviated hydrometer method, which, instead of 12 h for the standard method, requires about 3 h to complete. Twenty-four soil samples of varying textural classes determined by the standard method were reprocessed for particle-size and textural-class determination using an abbreviated hydrometer method. Results of the methods comparison showed that the textural class from the abbreviated method matched that of the standard method in only 10 of 24 samples and that the abbreviated method over-estimated the amount of total sand in the soil sample. The abbreviated method was reasonably accurate in comparison to the standard method with respect to percentages of clay and silt. Based on this comparison, the time savings gained with the abbreviated method do not outweigh the lack of accuracy of particle-size determination with coarse-textured soils, but may be justifiable for fine-textured soils without a large fraction of sand-sized material.

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MEET THE STUDENT-AUTHOR

Lauren A. Williams-Caudle

I was born at Fitzsimmons Army Medical Center in Denver, Colo., to a career Army Officer. Since then I have lived all over the United States and abroad. I came to the University of Arkansas from Georgetown High School in Georgetown, Tex. on an Army ROTC scholarship. Two years into the program I broke my back and had to choose another career path. I decided to major in environmental, soil, and water sciences offered in the Department of Crop, Soil, and Environmental Sciences (CSES) after taking Dr. Wolf's Introduction to Environmental Science course. Dr. Wolf made the major sound interesting and this area of study was what I had been looking for since I have always loved the outdoors. I have participated in soil judging and the departmental student club. I also studied in Israel with Dr. Bacon and at the Scottish Agricultural College during Spring Break 2000.

Dr. Rutledge, pedologist in CSES, originally devised this project and the laboratory work was conducted under the supervision of Michelle Steele. The development of this research paper came under the direction of Dr. Brye, whom I got to know through the Soil Judging Team. I could not have completed this project without Dr. Brye's assistance. Dr. Brye has been a great mentor through the difficult scientific writing process. I graduated in May 2003 and I intend to stay in the area while my husband completes his degree.

INTRODUCTION

In soil science it is important to know the particle-size distribution when classifying a soil. The textural class is important in many aspects of soil science subdisciplines. Textural analysis can help to determine several soil properties. For example, to determine whether or not a septic system can be installed, the soil texture must be known in order to classify the soil so that drainage capability can be identified. Textural class is also used by the Extension Service to aid farmers and gardeners in determining soil fertility requirements for commercial farms or gardens.

There are several methods that can be used to determine particle size. The two most common and inexpensive methods are the pipet and hydrometer methods (Gee and Bauder, 1986). Both of these methods are based on Stoke's Law, which relates a particle's diameter and mass to the time required to fall out of suspension. In general, for the pipet method, a suspension is created by plunging soil mixed with a dilute dispersing agent. After a specified time after cessation of plunging, an aliquot of the suspension is removed from a certain depth in the suspension using a pipet. The aliquot is washed into a container, oven dried, and weighed to determine the mass of particles remaining in suspension at the specified sampling time.

The initial steps involved in the hydrometer method are similar to those in the pipet method. A suspension is created by plunging a mixture of soil with dilute dispersing agent, but, in contrast to the pipet method, after a specified time a hydrometer is lowered into the suspension and the density of the suspension is determined. The density measurement from the hydrometer is used to calculate the mass fraction of particulates remaining in suspension at the specified sampling time.

A method for soil particle-size analysis that required less time to accomplish than the standard methods, which require over 12 h to complete, would allow particle-size analysis and textural class determination to be made in a more timely manner. In addition, a similarly accurate but shorter method would allow more samples and/or more replicates to be processed in less time compared to the standard method. Therefore, the objective of this study was to evaluate the accuracy of an abbreviated hydrometer method for determining soil particle

size compared to a standard hydrometer method. We hypothesized that the abbreviated method would be reasonably accurate compared to the standard method in determining soil textural class and sand, silt, and clay fractions.

MATERIALS AND METHODS

Particle-Size Analysis

Twenty-four soil samples of varying textural class determined by the standard hydrometer method (Day et al., 1955) were chosen for repeat particle-size analysis by an abbreviated hydrometer method. The soil samples had previously been air dried, crushed, and sieved through a 2-mm mesh screen.

For the standard method, 40 g of oven-dried, sieved soil were mixed with 50 mL of Calgon solution (i.e., 10% sodium hexametaphosphate), diluted to 500 mL with distilled water and then allowed to soak for at least 10 min. After soaking, the solution was mixed with a motorized mixer for 5 min, added to a 1-L sedimentation cylinder, and further diluted with distilled water to the 1000-mL final volume. Once this process was completed, the samples were left overnight to equilibrate to a constant temperature. Once the solution reached constant temperature, the solution was mixed thoroughly with a plunger. A hydrometer was inserted into the suspension and the density was recorded at 4.5 min after plunging ceased. The hydrometer was removed, rinsed, and the density of the suspension was recorded again at 6 and 90 min and at 6 and 12 h after plunging ceased. Once all readings had been recorded, the suspension was passed through a 300-mesh sieve to retain the sand fraction. Once the sample was free of silt and clay, the remaining soil material was washed into a 500-mL beaker to decant off the organic matter. Once only the sand fraction remained, the sample was oven dried at 105°C overnight. The sample was then placed in a mechanical shaker and sieved for 5 min through a series of sieves with 50-, 100-, 250-, 500-, and 1000-µm diameter openings, which represent the very-fine sand, fine-sand, medium-sand, coarse-sand, and very-coarse sand classes, respectively. After sieving, the mass of the sand fraction retained on each sieve and that which passed through the finest-mesh sieve was recorded.

The percentages of sand in each class and the total sand in the original soil sample were determined from Equation [1],

$$\% \text{ Sand} = [(\text{mass of sand fraction} / \text{mass of soil sample}) * \text{moisture factor}] * 100, \quad [1]$$

where the moisture factor was determined by oven-drying a separate 10 g of the initial air-dry soil so that the sand fraction could be expressed on an oven-dried basis. Before the silt and clay fractions could be determined, all hydrometer readings had to be corrected with a hydrometer reading in a soil-less blank. To calibrate the hydrometer in a soil-less blank, 50 mL of Calgon were added to a 1-L sedimentation cylinder and diluted to the 1-L mark with distilled water, and the cylinder was allowed to equilibrate overnight. The solution was plunged in the same manner as for the actual soil measurement and a hydrometer reading was recorded to represent a soil-less blank value. The percentages of clay were then determined from the density measurement at the 12-h mark after plunging ceased. The silt fraction was determined by difference from the total sand and clay fractions.

For the abbreviated method, the procedure outlined by Arshad et al. (1996) was followed. Fifty grams of oven-dried, sieved soil were added to an Erlenmeyer flask along with 50 mL of Calgon solution and the mixture was placed on a magnetic stirrer for 5 min. After stirring, the mixture was added to a 1-L sedimentation cylinder, filled with distilled water to the 1-L mark, and allowed to equilibrate to a constant temperature overnight. The following day, samples were plunged by hand. Hydrometer readings were recorded at 40 s and 2 h after plunging ceased. The hydrometer readings were corrected based on readings from a soil-less blank as was previously described for the standard method. The following equations were used to determine percentages of total sand and clay from the abbreviated method,

$$\% \text{ Silt} + \% \text{ Clay} = (R_{40\ sec} - R_{Blank}) / (\text{oven-dried soil weight in g}) * 100 \quad [2]$$

$$\% \text{ Clay} = (R_{2\ hr} - R_{Blank}) / (\text{oven-dried soil weight in g}) * 100 \quad [3]$$

$$\% \text{ Sand} = 100 - (\% \text{ Silt} + \% \text{ Clay}). \quad [4]$$

The percentages of silt were determined by difference using the results from Equations [2] and [3].

Textural-Class Determination

The soil textural class for each of the 24 samples was determined using an electronic version of the textural triangle accessed through a computer program (MSU, 2003). The program uses the values for total sand and silt as input variables, calculates the clay fraction by difference, and outputs the textural class.

Statistical Analysis

Linear-regression analysis was used to compare the percentages of total sand, silt, and clay from the abbrevi-

ated method (dependent variable) to those from the standard method (independent variable) (Minitab 13.31, Minitab Inc., State College, Penn.). If the percentages of total sand, silt, and clay from the abbreviated method matched exactly those from the standard method, we would expect the slope of the resulting regression line to be 1 with an R^2-value of 1.

RESULTS AND DISCUSSION

Comparison of Particle-Size Analysis

For the total-sand comparison, the relationship between the abbreviated method and the standard method was significant ($P \leq 0.001$). The resulting regression equation fit the total-sand data well ($R^2 = 0.95$). However, due to a slope of 0.71 and an intercept of 26.6, the abbreviated method resulted in an over-estimation of the total-sand fraction compared to the standard method, where the over-estimation was greatest at small sand fractions (Fig. 1). The accuracy of the abbreviated method could be improved if multiple 40-s readings were conducted on each soil sample before allowing the samples to sit for the remaining 2 h.

Although not measured directly in either procedure, the relationship between the abbreviated and standard methods for silt was also significant ($P \leq 0.001$). The resulting regression equation also fit the silt data well ($R^2 = 0.92$). The slope of the regression line was the smallest, 0.63, of the three particle-size comparisons, which indicated that the abbreviated method underestimated the silt fraction, but the y-intercept was also the smallest of the three comparisons, 1.4 (Fig. 2).

The methodological comparison of the clay fraction yielded the best results. The linear relationship between the methods was significant ($P \leq 0.001$) as it was with the total-sand and silt comparison (Fig. 3). The regression line fit the clay data the best of the three particle-size comparisons ($R^2 = 0.97$). The slope of the regression equation was closest to 1 (i.e., 0.84) and the y-intercept was reasonably small at 4.3.

Comparison of Textural Class

In contrast to the particle-size comparisons, the abbreviated method was not sufficiently accurate compared to the standard method for determining soil textural class. Aside from the sub-classes of sand, the resulting textural class (from the percentages of sand, silt, and clay determined by the abbreviated method) matched the textural class from the standard method for only 10 of 24 soil samples; in other words, a matching efficiency of only 42% (Table 1). This result does not support the original hypothesis that the abbreviated method can be used in place of the standard method for determination of soil textural class.

Although the standard method is more accurate, the abbreviated method can be used in time-constraint situations or in situations where the coarse-soil fractions are either unimportant or do not need to be identified. Based on the resulting over-estimation of the sand fraction by the abbreviated method, it is recommended that several trials be conducted on each sample and that an average be used to increase accuracy for the total-sand fraction when the abbreviated method is used. In addition, multiple trials could be conducted to reduce the slight under estimation of the silt and clay fractions. This experiment also demonstrated that the abbreviated method was less than 50% accurate for identifying the soil textural class. With this study, it was shown that the abbreviated hydrometer method for particle-size determination could be a reasonably accurate and time-saving procedure for determining the fine-textured soil particles (i.e., silt and clay), but it is less accurate for determining the fraction of coarser soil particles (i.e., total sand) and the resulting textural class.

ACKNOWLEDGMENTS

Appreciation is extended to the Dale Bumpers College of Agricultural, Food and Life Sciences Undergraduate Research Award Program and the Arkansas Agricultural Experiment Station for providing financial support.

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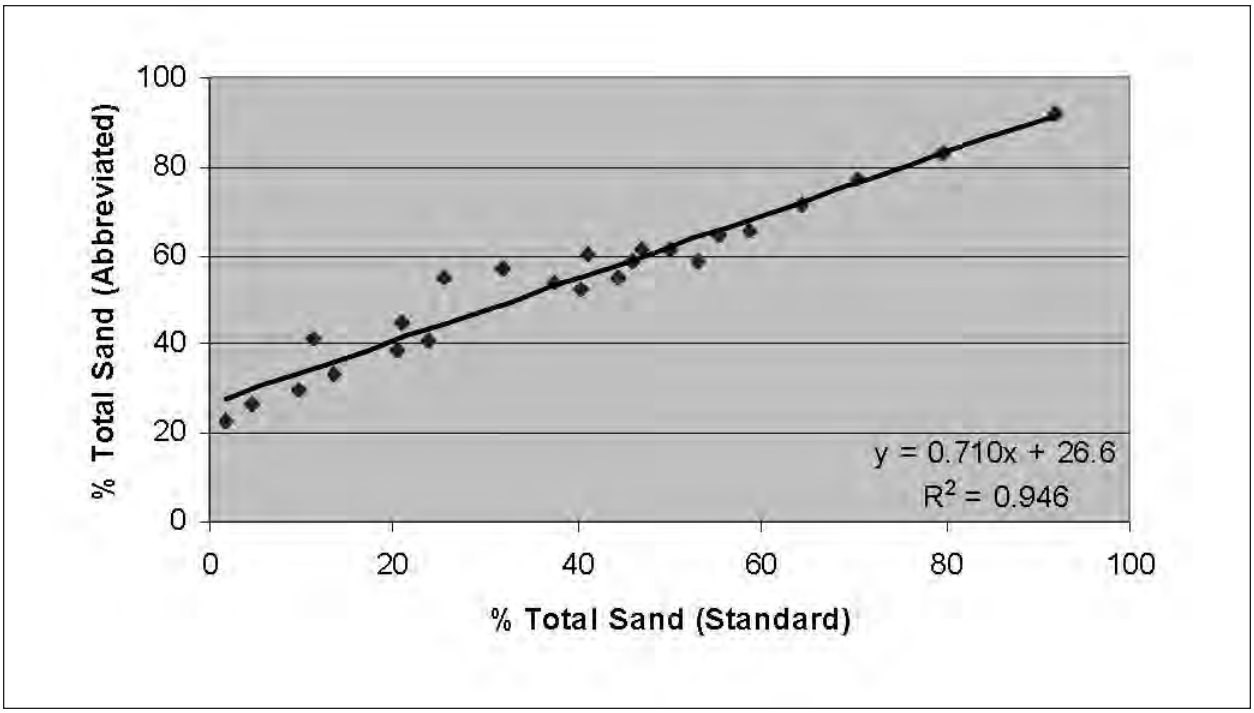

Fig. 1. Relationship between the percentage of total sand from the abbreviated and standard hydrometer methods for particle-size determination.

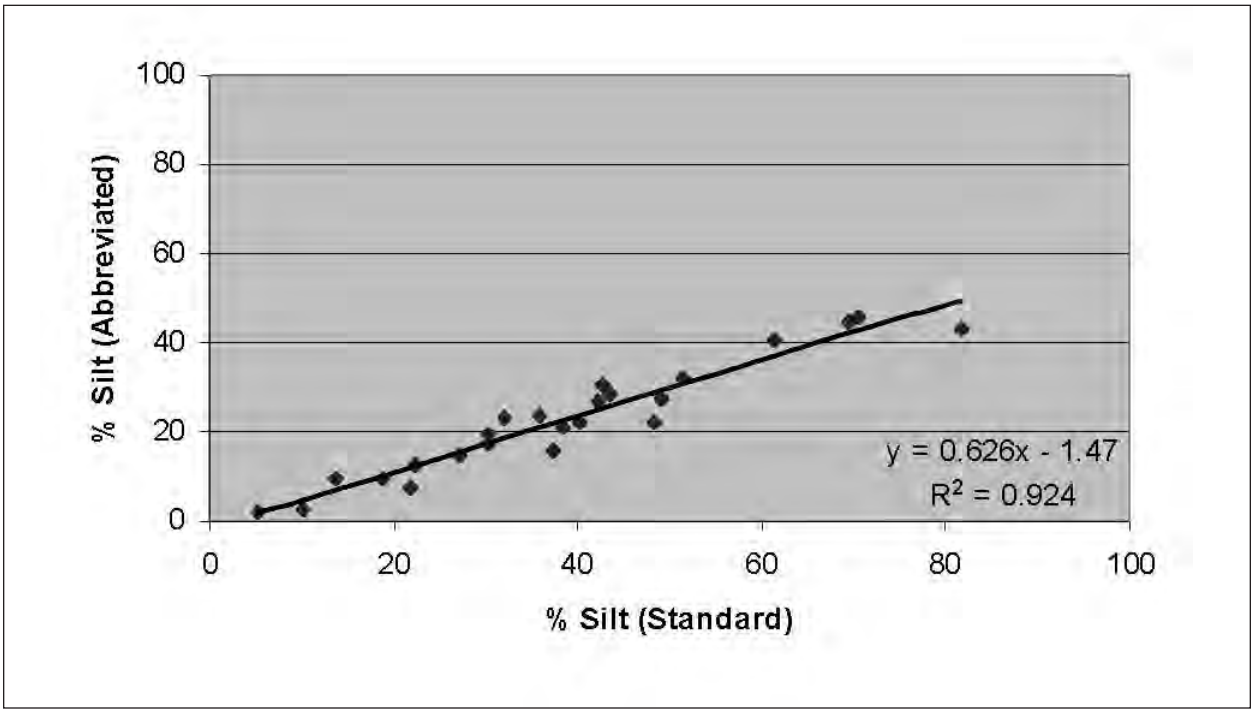

Fig. 2. Relationship between the percentage of silt from the abbreviated and standard hydrometer methods for particle-size determination.

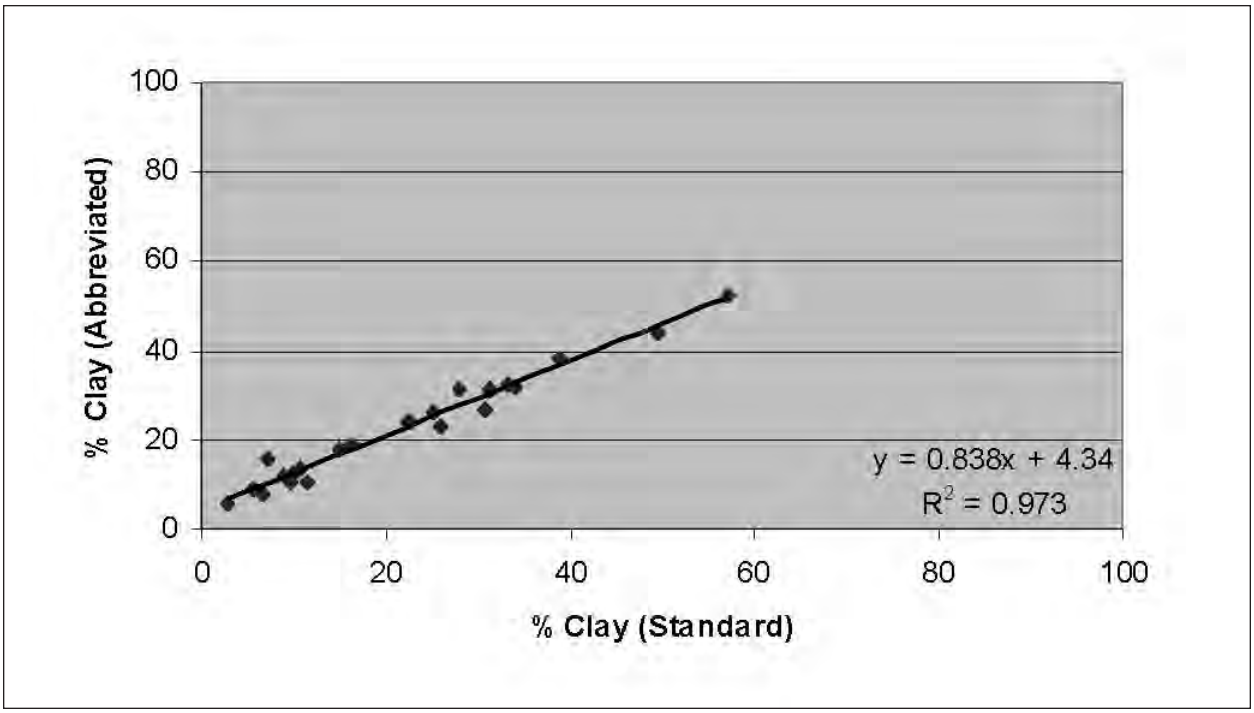

Fig. 3. Relationship between the percentage of clay from the abbreviated and standard hydrometer methods for particle-size determination.

Table 1. Soil textural classes from the standard and abbreviated methods.

	Textural class	
Sample ID	Standard method	Abbreviated method
10431	Loam	Sandy loam
10434	Clay	Clay
10442	Loam	Loam
10443	Loam	Sandy clay loam
10446	Very fine sandy loam	Sandy loam
10455	Clay loam	Sandy clay loam
10465	Silty clay loam	Clay loam
10473	Silt loam	Loam
10484	Silt loam	Loam
10488	Clay loam	Clay loam
10489	Clay loam	Sandy clay loam
10532	Fine sandy loam	Sandy loam
10535	Fine sandy loam	Sandy loam
10541	Silty clay loam	Clay loam
10548	Clay loam	Clay
10559	Silt	Silt
10563	Loam	Sandy clay loam
10570	Sandy clay	Sandy clay loam
10575	Sandy loam	Sandy loam
10576	Coarse sand	Sand
10581	Sandy clay	Sandy clay loam
10586	Sandy clay	Sandy clay loam
10620	Loam	Sandy loam
10667	Loamy fine sand	Loamy sand

Instructions for authors

STYLE GUIDELINES

Discovery uses *Scientific Style and Format: The Council of Biology Editors Manual for Authors, Editors, and Publishers* as its style manual. Refer to the latest available edition of the CBE manual for any questions not covered in these guidelines.

For research in disciplines where professional journals use style guides that differ significantly from the CBE, please consult the *Discovery* managing editor for guidance. You may also use this issue of *Discovery* as a style guide.

Writing style should be consistent with professional journals in the student's discipline. However, articles will be reviewed and read by people with varied backgrounds. Therefore, authors should avoid scientific jargon and should use a style and vocabulary that can be understood by any educated reader.

FORMAT

Report measurements in metric and other standard scientific units. Units or symbols that are likely to be unfamiliar to a general readership should be defined.

Figures must be submitted as electronic TIFF files. All figures should be generated by authors in the original charting software at 300 dots per inch (dpi) at a standard figure width of 4 inches (select "constrain proportions" and height will default proportionally), and exported or saved as TIFF files, if the original charting software offers a TIFF file format. If not, save all figures (and scanned photos) as TIFF files out of Adobe PhotoShop or other similar software. Select the "Save for Mac" byte order option if available in the original charting software "save as" function. If saving as TIFF files out of Adobe PhotoShop, select the "Save as" function to access the TIFF file format and specify the "Save for Mac" option in the byte order dialogue box. The size of figures will be adjusted by the editor to fit the page layout.

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

For tables, it is important to use tabs between columns of data instead of spaces.

Microsoft Word is the preferred text format, although WordPerfect will be accepted.

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

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

PARTS OF THE MANUSCRIPT

The title page should include the following:

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

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

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

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

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

The *Literature Cited* section lists the complete references corresponding to those cited in the text. Within the text, references are indicated by (Last Name, Year), e.g., (Jones, 2000) (Smith and Jones, 2000) (Brown et al., 2000) The complete citation is listed at the end of the manuscript in alphabetical order by the first author's last name. Multiple citations of the same author are listed chronologically or by order of reference in the text if dated the same year.

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Ryugo, K. 1998. Fruit Culture: Its Science and Art. John Wiley and Sons, London.

Internet URL citations are written as follows:

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Submit two copies of a printed manuscript on 8.5 x 11-in. paper, with double-spaced, 12-pt. text on one side, and an electronic file on Zip 250 mb disk or as an e-mail attachment to <cmromund@comp.uark.edu>. Mail or deliver to *Discovery*, Communication Services Unit, 110 AGRI, University of Arkansas, Fayetteville, AR 72701.

Also submit a brief personal biography (refer to past issues of *DISCOVERY* for style and length) and phone the Communication Services office at (479) 575-5647 to arrange an appointment to have your photo taken for the magazine. Include a cover letter signed by a faculty sponsor or mentor and all authors. Unless otherwise indicated, the editor will correspond with the first author for revisions, approval of proofs, etc. Note: First author (student) must include a current and a forwarding e-mail address (or phone number) for contact outside the school year.

Length should be limited to about 2000 words, but no minimum or maximum length is required.

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- Publish with acceptable revision
- Revise and resubmit
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