Effects of grain by-products as supplements for stocker cattle grazing bermudagrass

Tyler E. Davis*, Elizabeth B. Kegley†, Kenneth P. Coffey§, Wayne K. Coblentz‡, Robin K. Ogden§§, and J. A. “Pete” Hornsby‡‡

ABSTRACT

Two experiments were conducted to compare corn, dried distillers’ grains (DDG), and pelleted soybean hulls (SH) as supplements for cattle grazing bermudagrass. In Exp. 1, 66 crossbred steers (306 ± 3.2 kg) were stratified by weight and allotted randomly to six 2.4-ha bermudagrass pastures for a 107-d study. One of three supplement treatments (corn, DDG, or SH) was assigned randomly to each pasture group and was offered at 0.5% (as fed) of body weight. Calves were weighed at 28-d intervals and supplement was adjusted after each weigh period. In Exp. 2, five ruminally cannulated steers grazed bermudagrass pasture and were individually fed supplements (corn, DDG, or SH) at 0.5% of body weight in a 3 x 3 replicated, incomplete Latin-square design with a 14-d adaptation and a 5-d sampling period. In Exp. 1, supplementation with DDG and corn increased (P < 0.04) the average daily gain compared to supplementation with SH (0.89, 0.87, and 0.74 kg for DDG, corn, and SH, respectively). In Exp. 2, in situ dry-matter-disappearance kinetic measures of bermudagrass were not affected by type of supplementation. The potential extent of digestion for DDG (93%) was lower than for corn (97%, P = 0.01) and SH (96%, P = 0.06). Supplementation with corn or DDG at 0.5% of body weight improved the gain of stocker cattle grazing bermudagrass compared to supplementation with SH, but these differences were not explained by differences in bermudagrass degradation kinetics.

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INTRODUCTION

Arkansas ranks 16th in the United States in cattle and calf numbers with approximately 1.8 to 1.9 million head. These cattle have a cash value of more than $430 million and a total impact of $1.4 billion on the Arkansas economy (UACES, 2005). Additionally, Arkansas has approximately 420,000 stocker cattle and compared to data over past years, the number of stocker cattle continues to rise (USDA, 2005). Stocker calves are weaned beef calves (typically 136 to 272 kg) that graze forage generally and are provided supplements of grain to provide additional energy, protein, and minerals to achieve desirable gains.

The type of feed and its cost play a role in the profitability of the enterprise; therefore, new sources of low-cost highly nutritious supplemental feeds are constantly being sought. With the increasing number of ethanol plants (Tjardes and Wright, 2002) in the United States due to a demand for less dependency on foreign oil and more environmentally friendly energy, there is also an increasing amount of by-products. These by-products, known as distillers’ grains, remain following the production of ethanol mostly from corn and contain high concentrations of protein and energy (Tjardes and Wright, 2002). However, phosphorus (P) concentrations in this by-product are well above cattle requirements and could potentially cause cattle-health (possible formation of urinary calculi) and environmental (P in run-off) problems (Tjardes and Wright, 2002). Distillers’ grains have been used as an economical feed source for feedlot and dairy cattle for years; however, with this increasing supply, they may now be a more economical supplement for use in grazing animals.

There has been limited research investigating using distillers’ grains as a supplement for calves grazing bermudagrass \( \text{Cynodon dactylon} \). Bermudagrass can be

MEET THE STUDENT-AUTHOR

Upon graduation from Ashdown High School in May 2002, I enrolled at the University of Arkansas. My dream of being a “Razorback” was finally fulfilled. I was awarded the Honors College Academy Scholarship and the Alumni Society “Roads” Scholarship. In addition to these scholarships, I was fortunate to receive the Brandon Burlsworth Memorial Scholarship in 2005.

While on campus I have been actively involved with the Student Alumni Board, Pre-Dental Society, Associated Student Government, and served as President of the Block and Bridle Club. As a sophomore, I began working part-time on the Division of Agriculture Stocker-Receiver Beef Unit under the direction of Pete Hornsby and my mentor, Dr. Beth Kegley.

Being from a strong agricultural background, I have always possessed a passion for agriculture. My family owns and operates a commercial cow-calf and stocker cattle operation in rural Little River County, and I also own a herd of registered Angus cattle. This research project presented me with an opportunity to expand my knowledge and explore other aspects of the cattle and agricultural industries.

I am a senior majoring in animal science and will graduate with honors in May 2006. I have been accepted at the University of Tennessee-Memphis College of Dentistry and plan to specialize in pediatric dentistry.

I would like to thank especially Dr. Beth Kegley for her support and guidance as well as Dr. Ken Coffey for his research expertise. Additionally, I would like to thank Doug Galloway, Pete Hornsby, and Robin Ogden for their assistance during my research trial. All of those involved are greatly appreciated.

Tyler E. Davis
found on many farms in Arkansas; it is estimated that over 2 million acres of bermudagrass exist in the state (UACES, 2006). Although soil fertility, rainfall, and maturity affect bermudagrass nutritive value, the high fiber content of bermudagrass limits optimal calf growth. Calves grazing bermudagrass generally respond positively to supplementation of energy provided from grains. Yet, high levels of starch-containing grains, such as corn, decrease forage intake and fiber digestion of forage-based diets if supplemented at higher levels (Garcés-Yepez et al., 1997). Soybean-hull pellets are a locally available by-product of milling soybeans; these pellets are low in starch and thus provide energy without possible negative impacts on fiber digestion (Galloway et al., 1993). A comparison of these feedstuffs (distillers’ grains, soybean-hull pellets, and corn) in growing cattle would provide important information for Arkansas producers and allow them to make more informed and economical supplementation choices. Therefore, objectives in this study were to determine the impact of providing cattle with supplements of corn, dried distillers’ grains (DDG), or soybean-hull pellets (SH) on growth performance, blood metabolites and ruminal-forage degradation kinetics in cattle grazing bermudagrass pastures.

**MATERIALS AND METHODS**

There were two phases of this project. Experiment 1 was conducted to evaluate corn, DDG, and SH as protein and energy sources for stocker cattle grazing bermudagrass as well as to determine if suplementation influenced blood metabolites. Experiment 2 was conducted to evaluate the impact of these same supplements on ruminal digestibility of these supplements as well as bermudagrass pasture samples, using an in situ procedure. All procedures involving steers were approved by the University of Arkansas Animal Care and Use Committee.

**Experiment 1:** Sixty-six crossbred steers (initial body weight averaged 306 ± 3.2 kg) were stratified by weight and allotted randomly to six 2.4-ha pastures at the University of Arkansas Stocker-Receiving Unit near Savoy, Ark. on 11 May 2005. Calves had ad libitum access to fresh water and were monitored daily for morbidity. One of three supplementation treatments (corn, DDG, or SH) was assigned randomly to each pasture group and steers were offered these supplements at 0800 h daily at a rate of 0.5% (as fed) of body weight. Pastures were predominately bermudagrass (14.7% crude protein, 68% neutral digestible fiber, 32% acid detergent fiber, 0.39% P) and averaged 6,377 kg/ha of available forage over the 107-d study. Calves were weighed at 28-d intervals and the amount of supplement offered was adjusted after each weigh period such that calves were offered 0.5% of body weight; any supplement refusals were recorded daily. Supplement samples were collected every 28 d and were analyzed for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and P on a dry-matter (DM) basis.

Cattle were weighed at the beginning and end of the trial on two consecutive days, and interim weights were taken every 28 d. Blood samples were collected on d 0, 28, 56, 84, and 107 via jugular venipuncture with vacuum tubes. These samples were stored on ice after collection and centrifuged at 1,200 x g for 20 min for separation of serum or plasma; then serum or plasma samples were also taken in tubes with EDTA (BD Vacutainer®, Franklin Lakes, N.J.) and analyzed with a colorimetric assay (L-Type UN kit, Wako Chemicals USA, Inc., Richmond, Va.). Samples for serum P concentrations were also taken in tubes with the clot activator; serum was de-proteinized with 10% trichloroacetic acid and then P was determined with a colorimetric procedure (Bodine and Purvis, 2003). Samples for plasma non-esterified fatty acid (NEFA) concentrations were taken in tubes with EDTA (BD Vacutainer®). Plasma was analyzed with a commercial colorimetric assay (NEFA-C kit, Wako Chemicals USA, Inc., Richmond, Va.).

Fecal grab samples were taken from four calves per pen on d 84 and 107 and stored frozen to examine fecal P concentrations. Fecal material was later thawed, dried, ground to pass through a 1-mm screen of a Wiley Mill, sub-sampled, wet-ashed with nitric acid, and P determined with a colorimetric assay (Bodine and Purvis, 2003). Additionally, forage availability was measured every 28 d with a calibrated, rising disk meter, and grab samples were taken and combined in a composite sample to determine CP, NDF, ADF, and P.

Steer weights, average daily gain, blood metabolites, and fecal P were statistically analyzed using PROC MIXED of SAS (SAS Inst., Cary, N.C.). The experimental unit was a pen. A repeated statement was used for blood data.

**Experiment 2:** In a replicated incomplete 3 x 3 Latin square design, five ruminally cannulated crossbred (Gelbvieh x Angus x Brangus) steers (initial weight averaged 794 kg) grazed a bermudagrass pasture (1.46 ha) and had ad libitum access to fresh water. Steers were weighed at the beginning and end of each period. Each period consisted of 19 d, with 14 d of supplement adaptation followed by 5 d of in situ procedure. Period 1 began on 28 June 2005. Steers were caught daily at 0800 h and fed each of the supplements used in Exp. 1 (Table 1) at 0.5% (as fed) of body weight.

Bermudagrass was collected immediately prior to Period 1 of in situ collection from the same pasture the steers were grazing. In situ procedures were used as
There were no effects of supplement type on serum urea-N (Fig. 2). Steers supplemented with DDG had the greatest serum urea-N concentrations, steers supplemented with SH had intermediate concentrations, and steers supplemented with corn had the lowest concentrations of serum urea-N (P < 0.01). This was due to the greater amount of CP that the DDG and SH contained compared to corn (Table 1). This excess protein was degraded in the rumen and the ammonia was absorbed across the rumen wall. The liver detoxified the ammonia by forming urea that circulates in the bloodstream until being excreted in the urine (Church, 1988). Because of the high level of CP in the bermudagrass, none of these steers, even those supplemented with corn, should have been deficient in protein. All of these serum urea-N concentrations are considered high for cattle, and the concentrations for the steers supplemented with DDG were approaching levels that may cause decreased fertility for heifers of breeding age (Elrod and Butler, 1993).

Concentrations of plasma NEFA (Fig. 3) were not different among treatment sources. Plasma NEFA concentrations are increased when fat stores are being metabolized. Concentrations of NEFA were greatest on d 0, before supplementation started, and were low for the remainder of the experiment, indicating that energy was not limiting for these growing steers (Clarenburg, 1992).

There was a treatment x day interaction (P < 0.05) for serum-P concentrations (Fig. 4). Serum-P concentrations were consistent until d 107, when steers supplemented with corn had lower concentrations of serum P than steers supplemented with DDG or SH.

There was a main effect of supplement source and day on fecal-P concentrations (P < 0.003). Steers supplemented with DDG had the greatest fecal-P concentrations (0.84%), corn supplemented steers were intermediate (0.70%) and did not differ from that of steers supplemented with SH who had the lowest concentrations of fecal-P (0.66%) (data not shown). These results were expected due to DDG having a greater concentration of P, corn being intermediate, and SH containing the lowest P concentration. Fecal-P concentrations also varied by day, with concentrations on d 84 (0.70%) being lower than concentrations on d 107 (0.84%). This probably reflected the increased supplement intake during this last month of the study and also was due to an increased concentration of P in the bermudagrass during this last period due to regrowth after a rain event.

Experiment 1: Steer weights were not different until d 107, when steers supplemented with corn and DDG weighed more than steers supplemented with SH (P < 0.04) (Fig. 1). Average daily gains of steers supplemented with corn (0.87 kg) and DDG (0.89 kg) were greater than average daily gains of steers supplemented with SH (0.74 kg; P < 0.04) for the 107-d trial. These lower average daily gains in steers supplemented with SH differ from the results of Anderson et al. (1988) and Garces-Yepez et al. (1997). They reported similar average daily gains for cattle supplemented with corn versus SH. In the current study, supplement type did not affect forage availability in the pastures, and forage availability was never limiting (minimum observed was 3,080 kg DM/ha).

RESULTS AND DISCUSSION

Experiment 1: Steer weights were not different until d 107, when steers supplemented with corn and DDG weighed more than steers supplemented with SH (P < 0.04) (Fig. 1). Average daily gains of steers supplemented with corn (0.87 kg) and DDG (0.89 kg) were greater than average daily gains of steers supplemented with SH (0.74 kg; P < 0.04) for the 107-d trial. These lower average daily gains in steers supplemented with SH differ from the results of Anderson et al. (1988) and Garces-Yepez et al. (1997). They reported similar average daily gains for cattle supplemented with corn versus SH. In the current study, supplement type did not affect forage availability in the pastures, and forage availability was never limiting (minimum observed was 3,080 kg DM/ha).
However, Galloway et al. (1993) showed a decrease in bermudagrass hay intake and NDF digestion when cattle were supplemented with corn at 0.5% of body weight.

Ruminal disappearances of supplements were different among sources, with DDG having the greatest A fraction, corn intermediate, and SH having the smallest (P < 0.01) (Table 2). Soybean hulls had the greatest B fraction (P < 0.01), corn was intermediate, and DDG the smallest. Rate of disappearance (k_d) did not differ between DDG and SH, yet both rates were lower than that of corn (P < 0.01). Lag times were greatest (P < 0.01) from SH, intermediate from DDG, and smallest from corn. The potential extent of ruminal disappearance was greater for corn (P < 0.03) than for DDG with SH being intermediate and not different from either corn or DDG. However, all potential extents of ruminal disappearance were greater than 92%.

In conclusion, supplementation with corn or DDG at 0.5% of body weight improved average daily gains of stocker cattle grazing bermudagrass compared to supplementation with SH. However, in situ disappearance of bermudagrass was not different when these supplements were fed at the rate of 0.5% of body weight daily. All these supplement types produced desirable rates of gain for stocker cattle grazing bermudagrass in Arkansas.

ACKNOWLEDGMENTS

Financial support for this project was provided by the Arkansas Department of Higher Education Student Undergraduate Research Fellowship (SURF) and a Dale Bumpers College of Agricultural, Food and Life Sciences Honors College Research Grant.

LITERATURE CITED


Table 1. Nutrient composition of supplements fed to steers grazing bermudagrass pasture.

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Table 2. In situ disappearance kinetics of bermudagrass forage and supplements for steers grazing bermudagrass pasture in Exp. 2.

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<th>Fraction U</th>
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<th>Kds</th>
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<table>
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<th></th>
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1Fraction A = immediately soluble fraction, B = fraction disappearing at a measurable rate, and U = undegraded fraction
2Kd = ruminal disappearance rate
3Calculated as (100-U)
4Standard error of the mean
**P < 0.01
*P < 0.05

Fig. 1. Effect of supplement source on performance of steers grazing bermudagrass pastures in Exp. 1 throughout a 107-d trial. DDG = dried distillers’ grains; SH = soybean hulls.
Fig. 2. Effect of supplement source on serum urea-N concentrations from steers grazing bermudagrass pastures in Exp. 1. DDG = dried distillers’ grains; SH = soybean hulls.

Fig. 3. Effect of supplement source on plasma NEFA concentrations from steers grazing bermudagrass pastures in Exp. 1. DDG = dried distillers’ grains; SH = soybean hulls.
Fig. 4. Effect of supplement source on serum-P concentrations from steers grazing bermudagrass pastures in Exp.
1. DDG = dried distillers' grains; SH = soybean hulls.
Adventitious shoot propagation and cultural inputs in nursery production of a primocane-fruiting blackberry selection

Kimberley Dennis*, John R. Clark†, and James A. Robbins§

ABSTRACT

Studies were conducted from January to October 2005 to determine the effect of root-cutting length on adventitious shoot yield and the management practices necessary to produce nursery-quality primocane-fruiting blackberry plants. The first portion of the study measured the average number of shoots produced from 7.6 cm- and 15.2 cm-long root cuttings of APF-44 blackberry—a primocane-fruiting genotype from the University of Arkansas breeding program. Cuttings were forced in a shallow bin containing a soilless potting medium. The average number of shoots per root cutting from 7.6 cm- and 15.2 cm-long root cuttings averaged 1.6 and 2.7 shoots per root cutting, respectively. Rooting percentage for collected shoots was nearly 100% regardless of root-cutting length source. A qualitative comparison of shoots from the two root lengths was similar. The latter part of the study included various treatments on the rooted shoots that might affect the productivity and quality of the final product intended for nursery sales in early fall. With the aim of producing a flowering/fruiting shrub by late September, three treatments were applied: pot dimension, fertilizer rate, and shoot tipping. Fertilizer rate had the greatest impact of all treatments with the higher rate producing larger and more attractive plants. Above-normal summer/fall temperatures may explain lack of fruiting on APF-44 blackberries, but the dimension and size of some plants provided a portion of the intended aesthetic.

* Kimberley Dennis graduated in December, 2004 with a B.S. in horticulture.
† John R. Clark, faculty co-sponsor, is a professor in the Department of Horticulture.
§ James A. Robbins, faculty co-sponsor, is extension ornamental-horticulture specialist, Arkansas Cooperative Extension Service.
INTRODUCTION

Blackberries are a fruiting shrub in the genus *Rubus*, the same genus as raspberries; thus, cultivation of the two plants is very similar (Pritts and Handley, 1991). Traditional propagation methods of blackberries include tip layering, suckering, leaf-stem cuttings, tissue culture, and root cuttings (Caldwell, 1984). These techniques are often used but all have some limitations for propagators. Root cuttings, of all options, can be the most economical and timely way to propagate blackberries and the University of Arkansas uses root cuttings for nearly all blackberry propagation (John R. Clark, personal communication). Based on a study by University of Arkansas undergraduate, Ellen Thompson (Thompson et al., 2004), a “simple modification” to the traditional root-cutting propagation method led to increased propagule yield and rooting success. However, cultivar effects were sometimes significant. This modification of propagation technique allows for space-limited and/or greenhouse production and greatly decreases the time and monetary investment associated with traditional methods. Thompson’s study was based on precedents of a Swiss *Rubus* propagation system, and the findings were supported by the idea that forcing multiple adventitious shoots from a single root cutting increases the success and number of blackberry daughter plants. Moreover, the closely related raspberry is propagated in this way with similar results (Pritts and Handley, 1991).

The advent of primocane-fruiting blackberries, first introduced by the University of Arkansas breeding program in 2004, provides for potential increases in fruit yield and extends the growing season well into early fall (Clark et al., 2005). With this in mind, it might be possible to produce a nursery-quality plant that could produce fruit for home-gardeners into the autumn months. Moreover, the ornamental qualities of APF-44 blackberry specifically are conducive to a nursery’s aesthetic requirements; the plant is observed to have relatively short internodal length and a “bushy” mounded shape, and produces flowers and fruit in early September through October (John R. Clark, personal communication). Management practices to enhance these qualities—mid-summer tipping, fertilizer rates, and pot dimension—are investigated in this study.

MATERIALS AND METHODS

Experiment I: Adventitious shoot propagation

Root cuttings of two sizes, 7.6 cm and 15.2 cm, were evaluated in greenhouse conditions for propagule yield from January to April 2005. The APF-44 blackberry root cuttings used in propagation were collected from the University of Arkansas Fruit Substation, Clarksville, in late fall, 2004 (APF-44 is a breeding selection and is not released to the public nor is it an item of commerce). The diameter of the roots averaged approximately 5.3 mm. After one month of cold storage, the root cuttings were positioned in drainable plastic bins filled with LC1® soil-less potting mix (SunGro Horticulture; Alberta, Canada) to the fill line. Root cuttings were posi-
tioned horizontally at a depth of 2.5-3.5 cm below the medium surface. Greenhouse temperatures were maintained at a minimum temperature of 20°C and a maximum temperature of 25°C. Cutting bins were watered as needed. The experimental design was a randomized block of 10 replications of three root cuttings per replication. After the initial planting of root cuttings on 27 Jan. 2005, shoots began to appear on 1 March, and shoots continued to be harvested every 3-4 days as they grew to a length of approximately 5 cm with two partially expanded leaves. This harvest continued until 4 Apr. One hundred-three total shoots from the two root-cutting treatments were transplanted into individual Jiffy® peat pellets (Jiffy Co., Batavia, Ill.), and were subsequently placed under an intermittent mist system that misted the cuttings for 8 s every 10 min until shoots had rooted. Each shoot rooted in approximately 2.5 weeks. Shoot harvest continued until 4 Apr. when shoot production had significantly diminished. Data collection included percent shoot production per week, total number of shoots produced, shoot rooting success, and average number of shoots per root cutting.

Experiment II: Cultural inputs in nursery production of APF-44

Shoot cuttings rooted in peat pellets were transplanted into black plastic pots with the same media volume (9,000 cm³) but different pot dimensions: a “tall” Classic 1000-C® (25 cm top diam. x 23 cm tall) and a “squat” Classic 1200S-C® (28 cm top diam. x 19 cm tall) (Nursery Supplies Inc. (Chambersburg, Penn.). Transplanting began on 21 March and was completed on 4 April. On 30 April, potted plants were randomly arranged on an outdoor gravel pad. Plants were watered as needed using overhead impact sprinklers. Fertilizer rate and tipping treatments began on 13 May. The design was a randomized block of eight replications of eight of the following combination of treatments: 1) two pot dimensions (described above); 2) PolyOn 18-6-12, 8-9 month fertilizer topdress applied at a rate of 0.65 kg N/m² or 1.31 kg N/m²; and 3) and the tipping treatment applied to half of the plants in mid-summer. On 13 May, all plants were tipped to 30 cm in preparation for the growing season, and the fertilizer treatments were applied. On 15 July, all flowers and developing berries were removed and the tipping treatment was applied to the appropriate plants; these plants were again reduced to 30 cm in length by this tipping. By 12 Oct., the study was concluded and the following data were collected: 1) shoot growth index (GI); 2) shoot fresh weight; and 3) qualitative measurements—flowering and fruiting, plant shape, internodal length, leaf color, etc.

RESULTS AND DISCUSSION

Experiment I:

Root length had a significant effect on the number of adventitious shoots produced. The average number of shoots per root cutting for short (7.6 cm) and long (15.2 cm) root cuttings was 1.6 and 2.7, respectively. This finding supports results from an earlier study (Thompson et al., 2004). Shoot collection began approximately 4 weeks after roots were placed in the medium. Shoot collection was greatest in the first 4 weeks of shoot emergence; after this point, shoot production was significantly decreased (Fig. 1). A total of 72.3% of shoots from the 7.6 cm roots was harvested in the first 4 weeks. Similarly, 84.8% of shoots from the 15.2 cm roots had been harvested in the same time period. All shoots were of similar quality, and shoots were collected at the same point in development. The percent survival was nearly 100% (data not shown). Similar rooting percentages were reported previously (Thompson et al., 2004). These results suggest that this method also is successful for propagation of this blackberry genotype. The shoots grew vigorously in the peat pellets and rooted in approximately 1-2 weeks.

Experiment II:

Fertilizer rates of 0.65 kg N/m²and 1.31 kg N/m² had a significant effect on all plant growth parameters. Shoot fresh weights for plants grown at the low and high fertilizer rates were 155 and 400 gm, respectively (Table 1). Fertilizer rate also had an effect on the height of the plants; on average the plants with the lower fertilizer rate were 0.43 m tall versus 0.59 m for the higher rate (Table 1). In a similar way, average plant widths of the high-fertilizer-rate plants were greater than for those receiving the low fertilizer rate (Table 1). The plants that were not tipped averaged 0.47 m tall, whereas the plants that underwent the July tipping treatment were 0.55 m tall (non-significant difference) (data not shown). All of the plants that were not tipped had a one-dimensional growth habit, with shoots tending to fall over and grow horizontally, while the tipped plants were more spreading and attractive (data not shown). No treatment resulted in plants with consistently different numbers of flowers or fruits (data not shown). Qualitatively, the plants with the best shape, leaf color, and size were the two tipped treatments and the higher fertilizer rate; pot dimension did not cause any aesthetic disparity (data not shown).

The main objective of these studies was to determine the propagation and production methods necessary to produce nursery-quality plants of APF-44 in a timely manner. As previous studies have concluded, root length plays a significant role in forcing adventitious shoots.
Longer roots produce more shoots – this is expected as they are simply longer. On the other hand, analysis of these values indicates that per 15.2 cm of root length, the shorter root produces more shoots on average for a similar length of space (i.e., 2 – 7.6 cm roots can produce 3.2 shoots per 15.2 cm, whereas the 15.2 cm roots produced 2.7 shoots in this study). This finding is of potential value to propagators, who need thousands of shoot cuttings. For instance, 2000 7.6 cm roots cuttings with a total root cutting length of 15,200 cm should yield 3200 shoots. Likewise, 1000 15.2 cm of root cuttings with the same 15,200 cm total should yield 2700 shoots. On the other hand, shoot quality was the same for the two root lengths. In fact, shoot quality was maintained at an excellent level throughout the first portion of the experiment: all shoots rooted and indicated no nutrient deficiency. Thompson et al. (2004) noted the same observation with 'Apache', 'Arapaho', and 'Ouachita' blackberry cultivars.

Building upon an efficient propagation method, this study is the first to investigate plant management techniques that might create a nursery-quality, primocane-fruiting blackberry marketable to homeowners during autumn. After shoots had been rooted and potted and treatments were applied, disparities began to appear among the plants. Plants that were tipped in mid-summer and fertilized with the higher fertilizer rate displayed the intended mounded shape and short internodal lengths. On the other hand, the main objective of this portion of the experiment was not met; at the conclusion of the study, no plants had plentiful berry or flower displays. The negative effects of heat on flowering have been observed for APF-44, and this may explain the problem (John R. Clark, personal communication). Also, plants remaining in a juvenile state due to pruning may be less likely to flower (John R. Clark, personal communication). In this particular study, flowers were plentiful before the May flower/bud removal; after this time, plants rarely produced buds. Blackberry plants without berries in September are not marketable as a nursery-quality ornamental intended for autumn sale. Fortunately, it is possible that additional breeding might achieve more abundant blooming and increased heat tolerance for flowers and fruits.

Further studies might include use of an adult second-year plant, a different genotype identified with more abundant flowering in heat, or simply moving the study to a more temperate climate. The root cutting method was successful, and no improvements are suggested.

LITERATURE CITED


Table 1. Effect of fertilizer rate on nursery plant shoot and plant growth parameters of APF-44 blackberry (APF-44 is a breeding selection and is not released to the public nor is it an item of commerce).

<table>
<thead>
<tr>
<th>Fertilizer rate (kg N/m3)</th>
<th>Shoot fresh weight (g)</th>
<th>Shoot height (m)</th>
<th>Plant width 1 (m)</th>
<th>Plant width 2 (m)</th>
<th>GIz (m3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.65</td>
<td>155.3 b†</td>
<td>0.431 b</td>
<td>0.658 b</td>
<td>0.292 b</td>
<td>0.076 b</td>
</tr>
<tr>
<td>1.31</td>
<td>400.3 a</td>
<td>0.586 a</td>
<td>1.200 a</td>
<td>0.729 a</td>
<td>0.428 a</td>
</tr>
</tbody>
</table>

† GI = growth index. Calculated by the formula hr², where h is shoot height, r=0.5d, and d is the mean of two diameter measurements taken at 90° angle from each other.

†Mean separation by LSD, P<0.05.
Fig. 1. Dates of adventitious shoot collection from APF-44 blackberry.
Initial evaluation of novel preparations of *Bordetella avium* by determination of antibody-response titers

*Joel L. Gallagher*, Stacy E. Higgins†, Luc Berghman§, Billy M. Hargis‡

**ABSTRACT**

The efficacy of killed vaccines generally is not equal to live vaccines. However, due to safety and ease of production, they remain a vital part of controlling and preventing diseases. In this study, the immune response to four different vaccination preparation techniques for the agent of bordetellosis of turkeys, *Bordetella avium* (BA), was compared. Preparation/inactivation techniques included (1) formalin inactivation, (2) opsonization of formalin-inactivated BA, (3) buffered acetic-acid BA inactivation, or (4) opsonization of buffered acetic-acid-inactivated BA. Non-adjuvanted suspensions containing equal antigen mass were administered subcutaneously (0.2 mL) at day-of-hatch in all cases. For each treatment (N=40/treatment), plasma samples were obtained on d 6, 10, and 21. Specific antibody titer was determined by enzyme-linked immunosorbent assay (ELISA). Results were analyzed by percentage of responders, calculated by determination of sample-to-positive (S/P) ratio. At d 6, the formalin-killed vaccination caused the most rapid response with significantly higher S/P ratios than other treatments. At d 10 there were no significant differences between the treatments. By d 21, formalin-inactivated antigen produced the highest percentage of responders. In this preliminary experiment, neither buffered acetic-acid BA inactivation nor opsonization of inactivated BA antigen improved turkey poult responsiveness to this pathogen.

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† Stacy Higgins is a Ph.D. candidate in the Department of Poultry Science
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‡ Billy Hargis, teacher and faculty mentor, is a professor in the Department of Poultry Science.
INTRODUCTION

Bordetellosis is an extremely contagious poultry disease caused by the organism *Bordetella avium* (BA), a small, Gram-negative bacterium that colonizes in the ciliated epithelium of the trachea of young turkeys (poults). The disease is characterized by interference with the respiratory mucosa and symptoms include sneezing and ocuonal nasal emissions (Skeeles and Arp, 1997). Although it is difficult to determine the precise economic loss to the poultry industry because the disease is often coupled with mortality and morbidity due to secondary infections, estimates of economic losses due to reduced body weight, reduced feed efficiency, and animal mortality are in the millions of dollars each year (USDA ARS, 2002).

Once infection has become apparent, treatments for bordetellosis have focused chiefly on antibiotic treatment rather than preventative care. This approach is slowly being phased out as the industry is moving away from using antibiotics in animals intended for human consumption (USDA ARS, 2002). Additionally, the emergence of antibiotic resistance has become a major concern worldwide. Indeed, several years ago in a document outlining strategies against microbial antibiotic resistance, the European Union labeled the situation as a “public health priority” and addressed the problem by taking steps to restrict antibiotic use to serious humans and animal health problems only (Commission of the European Communities, 2001).

Various studies have focused on treating the problem only after it arises with such treatments as the use of niacin (Yersin, 1991) and a novel oxy-halogen formula (Pardue and Luiginbuhl, 1998), both of which are administered through drinking water. Prevention in the form of vaccination is certainly the most effective as well as economic treatment, but current vaccines are not consistently protective. One research team explored the idea of passive immunity by isolating serum and tracheal remnants from convalescent poults, which resulted in a small reduction of adherence of *B. avium* to tracheal mucosa (Arp and Hellwig, 1988). A temperature-sensitive *B. avium* mutant known as ART-VAX, given via eyedrop/oral and spray cabinet protocols, showed a reduced severity of lesions although it failed to prevent infection upon challenge (Houghten and Skeeles, 1987). A formalin-inactivated bacterin also failed to produce consistent and long-lasting results under a challenge model (Hofstad and Jeska, 1985).

One promising method of vaccine production takes advantage of immune complexes, which are formed during an immune response to a foreign antigen through the binding of highly specific antibodies to a specific antigen. Introducing immune complexes to lymphoid follicles is thought to promote the creation of germinal centers, which in turn leads to the proliferation of memory B cells. This can result in increased antibody pro-

MEET THE STUDENT-AUTHOR

After graduating from Ruston High School (La.) in 2002, I decided to attend the University of Arkansas and major in biology on the premedical track. Upon acceptance to the university, I was awarded the Chancellor’s Scholarship, and during my junior year I was awarded the Gilbert Premedical Scholarship. I am member of the American Chemical Society, Alpha Epsilon Delta, and Golden Key International. I am also active with Catholic Campus Ministries, where I lead a student group that provides aid and advocacy for the poor.

During the spring semester of my freshman year, I began working for Dr. Hargis with his research on probiotic treatments for *Salmonella* enteriditis. I started my research project examining the immunological responses to novel *Bordetella avium* vaccines two years later, and then applied for and was awarded a State Undergraduate Research Fellowship (SURF) for two consecutive years. After graduation, I plan to pursue a career in medicine, specializing in pediatrics.
dium (Nayak et al., 1999; Nie et al., 1997; Kunkl and Klaus, 1981).

Although the precise mechanism remains uncertain, studies that examined the effects of immune complexes made with immunoglobulin-E (IgE) hypothesized that the increased antigen-specific response is due to efficient absorption of the complexes into B-cells via a low-affinity IgE receptor located on the B-cell surface. They also observed that the complexes specifically led to an increase in the number of immunoglobulin-G (IgG)-secreting cells, leading to the possibility of the existence of a secondary mode of antibody response that occurs without the need for priming (Westman et al., 1997). Complexes composed of equal amounts of antigen and antibody or a slight surplus of antigen had greater success at generating memory B-cells than antigen alone. The constant portion of the antibody appeared to be the key; the variable portion of the antibody protein proved to be less effective (Klaus and Humphrey, 1978). Immune complexes may also play a role in controlling the release of antigens by increasing the amount of time that immune cells are exposed to the antigen (Sah and Chien, 1996). Additional studies have shown that immune complexes resulted in a stronger localized response compared to the uncomplexed controls (Levy et al., 2001).

This concept of immune complexes has been incorporated into the development of a vaccine for infectious bursal disease virus (IBDV). Field trials have proven somewhat successful. Jeurissen et al. (1998) found that virus detection in the IBDV/complex group was delayed for 5 d compared to the IBDV group, and that germinal centers were much more prevalent in the spleens of the IBDV/complex chickens, demonstrating higher activity of B-lymphocytes in the IBDV/complex group. Although both treatments lead to depletion of B-cells in the bursal follicles, the reduction was less severe at all time points in the IBDV/complex group (Jeurissen et al., 1998). Using reverse-transcriptase PCR to detect the presence of IBDV in trials with the vaccine, it was found that the viral load peaked at d 17 for the SPF chickens and d 21 for the maternally immune broilers, eventually disappearing altogether as seroconversion occurred (Ivan et al., 2005). Another research team concluded that the IBDV/complex vaccine can be administered quite successfully as early as d 1 despite the presence of variable levels of maternal antibodies (Haddad et al., 1997).

Another area of vaccine production that can be improved upon is the inactivation method of killed vaccines. They are usually created by growing the organism of interest, and then adding formalin to ensure the organism is killed prior to administration via injection. Numerous vaccines have been created in this fashion, ranging from the Salk polio vaccine to diphtheria and tetanus vaccines. However, formalin will often cross-link proteins on the surface of cells through hydroxymethylene bridges and thus modify the three-dimensional protein structure within 24-48 hours of exposure (Metz et al., 2004; Werner et al., 2000; Rappuoli, 1997). Therefore, the animals might produce antibodies to artificial epitopes formed by the crosslinks rather than antibodies that are protective against live organisms during an actual infection. It is necessary to preserve antigenic structure in order to allow an adequate amount of antigen to be present for an extended period of time on the surface of dendritic follicular cells. This leads to the synthesizing of a higher number of B-memory cells specific to that antigen and hence a stronger immune response (Regenmortel, 1992).

The tendency of formalin to alter antigenic epitopes has been shown in several studies (Metz et al., 2003; Nencioni et al., 1991), especially with pertussis vaccines for humans. Clearly, formalin has the potential to alter the epitopes in a detrimental fashion. A more natural deactivation method is needed in order to avoid the potential outcomes associated with the use of formalin. An ideal deactivating agent would retain intact bacterial epitopes while inactivating the pathogen. Therefore, the objective of this study was to manufacture a killed vaccine that could be administered with a single injection and produce a strong, long-lasting immune response.

**MATERIALS AND METHODS**

**Production of antibodies for immune complexes**

Turkey-origin antibodies (Ab) were generated for use in these studies. Briefly, turkeys were injected twice with inactivated BA at 6 ($10^{10}$ cfu) and 9 ($10^5$ cfu) weeks of age. Serum obtained 10 d after the final injection was used for this experiment. Agar gel immunodiffusion (AGID) assays were used to estimate the relative titers for standardization during the opsonization process.

**Vaccine production and administration**

Four groups of vaccines were created – two batches killed with 3% formalin and two additional batches killed with 10% acetic-acid. One formalin and one acetic-acid group were complexed with the harvested turkey antibodies (Ab), while the two remaining formalin and acetic-acid groups were left uncomplexed (Table 1). The acetic-acid was buffered with the addition of NaOH to a pH of 4.75, which is near the pKa of acetic-acid. To create the complexed vaccines, *B. avium* was killed using methods described above, and dilutions of Ab were combined with 100 µl of *B. avium* at a concen-
tation of $10^6$ cfu/ml on slides, stained with Trypan blue, and examined in a microscope. The least Ab dilution that displayed evidence of immune complexes with some loose bacteria present was selected for opsonization. Once the dilution was determined, the formalin-complexed and acetic-acid-complexed vaccines were prepared. A fifth group consisted of negative controls, which were injected with either a formalin or acetic-acid vehicle (0.2 ml/poult).

The vaccines were administered to poult's on the day of hatch by subcutaneous injection (0.2 ml/poult at 5.68 x $10^9$ cfu inactivated). Each group, consisting of 40 tagged poult's each, was kept in separate pens furnished with heat lamps, feed, and water arranged in similar locations in each pen. Blood samples were obtained at d 6, 10, and 21 using 22-gauge needles and 5-ml syringes. In order to minimize clotting, heparin was used at a concentration of 20 units/ml. Each sample was centrifuged at 1500 RPM for 10 min and the plasma was isolated and stored in 1.5-ml microcentrifuge tubes at −20°C.

Titer determination

The antibody titer of each group at each time point was determined using an ELISA. The 96-well plates for the test were prepared and stored according to Hopkins et al. (1988), using 0.1M carbonate/bicarbonate buffer rather than 0.05M. Each plate contained triplicates of seven identical positive dilutions of serum from the hyperimmunized birds and triplicates of a negative dilution composed of pooled sera from the control group samples. These positives and negatives served as internal standards in each plate (Table 2).

A preliminary assay was conducted with selected dilutions of unknown samples to determine the appropriate dilution factor. From this preliminary assay, it was determined that a dilution of 1:25 provided the most reliable results while maintaining a feasible development time. Therefore, each unknown sample was diluted 25-fold and placed as duplicates into the remaining wells on each plate. The results from each plate were normalized using the internal standards and the absorption values of each of the unknowns were averaged. These values represented the relative titers between the samples. These relative titers provided a numerical measure of the amount of antibody present. In order to put the results into a more easily understood value, the sample-to-positive (S/P) ratio of each sample was calculated according to the below equation:

$$\frac{(\text{Sample mean} - \text{NC}\bar{x})}{(\text{PC}\bar{x} - \text{NC}\bar{x})}$$

where NC\bar{x} represents the negative control mean and PC\bar{x} represents the positive control mean. It was decided to use the 1:16,000 positive dilutions because it gave absorption readings around 2 absorption units. Readings around 2 absorption units indicated that those samples were on a part of the standard curve graph that was less sigmoidal and more linear. Using a more linear portion of the graph is more useful for titer predication purposes. This technique is widely used in various immunological contexts (Hendrick et al., 2005; Nagy et al., 2002). S/P ratios can even act as a rough estimate of the level of antibody in the serum (Snelson, 2003).

The mean, standard deviation, standard error, and probability were calculated using SAS (SAS, Inc., Cary, N.C.) statistics software to determine significant differences among groups. Differences are reported at P<0.05.

RESULTS AND DISCUSSION

S/P Ratios

Those samples that displayed a positive S/P ratio were said to have had an immune reaction to the vaccines. Alternatively, those with a negative S/P ratio were said to have little or no reaction. The graphs for each day depicted both the average positive and total average S/P ratio for each group in order to compare both the averages of those that responded and the total averages of responders and non-responders alike.

Percent of Positive S/P Ratios

All groups reacted to the vaccines to some extent (Fig. 1). More birds initially reacted to the formalin-inactivated group (F) compared to the acetic-acid-inactivated group (AA). However, d 21, 50% of the acetic-acid group had responded. No obvious differences were evident until d 21. At d 21, percentages generated by both formalin groups (F/F+Ab) were higher than those induced by both acetic-acid groups (AA/AA+Ab), reaching levels commonly believed to be necessary for flock immunity. The formalin-complexed (F) group on d 21 had statistically higher coverage than either of the acetic-acid groups (AA/AA+Ab), although results were not statistically different than formalin inactivation (F) alone.

Day 6 S/P Ratios

It is possible that the negative values displayed in the S/P ratio averages could be a result of the presence of non-specific binding sites on the B. avium bacteria for the constant (Fc) portion of antibodies (Fig 2). However, this possibility was not evaluated in our study. Although all groups had some negative S/P ratio averages, the formalin group (F) was significantly higher than the acetic-acid-complexed group (AA+Ab) and not significantly different from the other two groups. The acetic-acid group (AA) and formalin-complexed group (F+Ab) were not significantly different than the other
groups. Of the birds that had a positive S/P ratio and hence did respond to the vaccine, no significance was observed in any of the groups.

Day 10 S/P Ratios

The averages on d 10, where intermediate responses were anticipated, were not elucidating. No significant differences existed in either the total averages or positive averages in any of the groups (Fig. 3). Due to the similarity of the formalin/formalin-complexed and acetic-acid/acetic-acid-complexed averages, especially the positive averages, it appears that the addition of immune complexes did nothing to aid in the immunological reaction to the vaccine.

Day 21 S/P Ratios

With regard to the positive S/P ratio averages, the formalin and formalin-complexed groups exhibited significantly higher averages than the acetic-acid-inactivated group. The acetic-acid-complexed group was not significantly different from any group. Including all birds increased the interpretability of these results. The formalin-complexed and formalin groups both featured significantly higher averages than the acetic-acid-complexed and acetic-acid groups (Figure 4).

The formalin-inactivated vaccines, which approached 80% positive antibody response, were superior to the acetic-acid-inactivated vaccines by d 21. Addition of immune complexes was not of any benefit. From this experiment, it does not appear that either opsonization or the acetic-acid method for inactivation of BA for vaccine generation offer improved methods for inactivated BA vaccination of turkeys.

Development of an improved vaccine for *Bordetella avium* would have implications on all species that are susceptible to infection by *Bordetella* species, including canines and humans. Improvement of killed vaccines in general would have repercussions for many of our current vaccines for various animal immunizations.

ACKNOWLEDGMENTS

This research was funded by a State Undergraduate Research Fellowship (SURF) from the State of Arkansas.

LITERATURE CITED


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**Fig 1.** Percentages of each group with positive S/P ratios at day 6, 10 or 21. Histograms with different letters are significantly (p<0.05) different. Abbreviation of treatments are as follows: AA = acetic-acid, AA+Ab = acetic-acid-complexed, F = formalin, F+Ab = formalin-complexed.
Fig 2. Averages of all S/P ratios and positive S/P ratios in each group as measured on day 6. Histograms with different letters are significantly (p<0.05) different. Abbreviation of treatments are as follows: AA = acetic-acid, AA+Ab = acetic-acid-complexed, F = formalin, F+Ab = formalin-complexed.

Fig 3. Averages of all S/P ratios and positive S/P ratios in each group as measured on day 10. Differences between groups were not significant (p>0.05). Abbreviation of treatments are as follows: AA = acetic-acid, AA+Ab = acetic-acid-complexed, F = formalin, F+Ab = formalin-complexed.

Fig 4. Averages of all S/P ratios and positive S/P ratios in each group as measured on day 21. Histograms with different letters are significantly (p<0.05) different. Abbreviation of treatments are as follows: AA = acetic-acid, AA+Ab = acetic-acid-complexed, F = formalin, F+Ab = formalin-complexed.
Table 1. Production process of the four vaccines and controls.

<table>
<thead>
<tr>
<th>Step</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grow B. avium (10⁷ CFU/ml)</td>
<td>Complexed Acetic acid AA+Ab (Ba + Ab)</td>
<td>Uncomplexed acetic acid AA (Ba only)</td>
<td>Complexed formalin F+Ab (Ba + Ab)</td>
<td>Uncomplexed formalin F (Ba only)</td>
<td>Acetic acid negative controls (saline + AA)</td>
<td>Formalin negative controls (saline + F)</td>
</tr>
<tr>
<td>↓↓↓↓↓</td>
<td>Kill using 10% AA @ pH 4.2-4.5 (AA)</td>
<td>Add 10% acetic acid @ pH 4.2-4.5 (AA)</td>
<td>Kill using 3% formalin (F)</td>
<td>Add 10% acetic acid @ pH 4.2-4.5 (AA)</td>
<td>Add 3% formalin (F)</td>
<td>Add 3% formalin (F)</td>
</tr>
<tr>
<td>↓↓↓↓↓</td>
<td>Add 3% formalin (F)</td>
<td>Add 3% formalin (F)</td>
<td>Add 3% formalin (F)</td>
<td>Add 3% formalin (F)</td>
<td>Add 3% formalin (F)</td>
<td>Add 3% formalin (F)</td>
</tr>
</tbody>
</table>

(Where AA = Acetic Acid, Ba = B. avium, F = Formalin, Ab = Antibodies)
Effects of tank mixes of MON 3539 and selected compounds in RoundupReady Flex® cotton – 2005

ABSTRACT

Field experiments were conducted in 2005 to evaluate potential weed control interactions when MON 3539 (glyphosate) was applied with several insecticides and a plant growth regulator to RoundupReady Flex® cotton. Applications were made at the 1-3 leaf stage, the 6-8 node stage, and at the 12-14 node stage. Different combinations of tank mixes were used in each of the three applications. In the first application, all plots received the same treatment: MON 3539 at a rate of 0.75 lb ae/a. A second application was made to evaluate crop injury. Only the MON 3539 + Dimate (dimethoate) mixture significantly increased crop injury 7 days after treatment two (DAT2) when compared with MON 3539 alone (20 vs. 13% injury). Bidrin (dicrotophos), Trimax (imidacloprid), Mustang Max (zeta-cypermethrin), Karate Z (lambda-cyhalothrin), Baythroid (cyfluthrin), Intrepid (methoxyfenozide), Steward (indoxacarb), Denim (emamectin benzoate), insecticides or Mepichlor (mepiquat chloride) plant growth regulator in combination with MON 3539 showed less than 8% crop injury at 7 DAT2, which was significantly less than MON 3539 applied alone (13% injury). Crop injury ratings were taken following a third application and only the MON 3539 + Mepichlor mixture significantly increased crop injury at 7 days after treatment three (DAT3) when compared with MON 3539 alone (13 vs. 5% injury). None of the remaining treatments in the third application significantly differed from that of MON 3539 alone. Weed control rating indicated that MON 3539 + Centric (thiamethoxam) significantly reduced weed control at 15 DAT2 when compared with MON 3539 alone (72 vs. 84% control). MON 3539 tank mixed with each of the following significantly differed from the 95% rating of MON 3539 alone at 14 DAT3: Bidrin at 75%, Centric at 72%, and Denim at 79%.

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† Gus Lorenz is a professor of Entomology and extension entomologist–IPM Coordinator, UACES.
§ Kyle Colwell is a program technician with the University of Arkansas Cooperative Extension Service in the Department of Entomology.
‡ Craig Shelton is a senior at Arkansas State University.
INTRODUCTION

RoundupReady® cotton, which is tolerant to glyphosate herbicides, requires over-the-top herbicide applications before the cotton plant reaches the 5-leaf growth stage. During this period, environmental conditions such as rain and wind can make these applications difficult. RoundupReady Flex® cotton cultivars provide the ability to make over-the-top applications after the 5-leaf growth stage with higher rates of glyphosate. RoundupReady Flex® cotton has been found to show “excellent tolerance to POST glyphosate applications up to the 14-leaf cotton growth stage at rates two to three times higher than the current use rate in RoundupReady cotton” (Keeling et al., 2004). The ability to apply glyphosate later in the season allows mixing with insecticides as well as combining with the plant growth regulator (PGR), mepiquat chloride to control plant height.

During the 2002 growing season, an estimated 10% of the total cotton crop was lost due to weed infestation of grasses and broadleaves. This equates to 130,000 bales lost out of a total of 1,300,000 bale yield potential. From 1,615,035 bales classed, the revenue lost in Arkansas was $130,000 with the assumed price reduced by $0.03 per pound of lint (Byrd, 2003). Currently in Arkansas cotton, glyphosate is used in preplant-burndown situations for annual grasses and broadleaf weeds. It is also used in postemergence applications for the control of emerged annual grasses, johnsongrass, and numerous other weeds, including cocklebur, sicklepod, pigweed, morningglory, prickly sida, velvetleaf, hemp sesbania, northern jointvetch, and smartweed (Scott, 2004).

Antagonism/synergism resulting from the tank-mixture of glyphosate products with various insecticides have become important considerations in recent years. It has become a serious question as to whether it is feasible for a grower to mix glyphosate with insecticides to save application time and money. The ability to apply herbicides over-the-top of cotton past the 5-leaf growth stage will create an opportunity for growers to reduce production costs by the combination of glyphosate and...
insecticides in a single operation (Mascarenhas and Griffin, 1997). This study investigate the mixing of various insecticides and a PGR, mepiquat chloride tank-mixed with glyphosate to determine any positive and/or negative effects.

**MATERIALS AND METHODS**

The experiment was conducted on Hooker Farms, Pine Bluff, Ark., (Jefferson County) in 2005. MON B2RF, a non-commercial Monsanto cultivar, was planted on 6 May. The planted field was subdivided into plots of four rows (38-inch spacing), 30-feet in length. Plots were set up in a randomized complete block with four replications. Treatments were made according to statewide threshold recommendation. Treatments were applied with a CO2 backpack applicator using a 4-row boom with Tee-Jet TXVS 6 nozzles on 19-inch spacing. Operating pressure was 40 pounds per square inch and volume applied was 10 gallons per acre. Three separate applications were made in this test. The first application was made 26 May at the 1-3 leaf stage. All plots were treated with MON 3539 (glyphosate) at a rate of 0.841 kg/ha (0.75 lb ae/a). The second application was made 14 June at the 6-8 node stage and consisted of MON 3539 alone as a control, or MON 3539 tank-mixed with selected insecticides or mepiquat chloride to determine the potential for crop injury (phyteneosis) and/or loss of weed control. Treatments included MON 3539 at 0.841 kg/ha (0.75 lb ae/a) alone or mixed with one of the following: Orthene (acephate) at 1.12 kg/ha (1 lb a/a), Bidrin (dicrotophos) at 0.56 kg/ha (0.5 lb ai/a), Vydate C-LV (oxamyl) at 0.529 kg/ha (0.471 lb ai/a), Dimethoate at 0.56 kg/ha (0.5 lb a/a), Trimax (imidacloprid) at 0.053 kg/ha (0.0469 lb ai/a), Centric (thiamethoxam) at 0.056 kg/ha (0.05 lb ai/a), Mustang Max (zeta-cypermethrin) at 0.028 kg/ha (0.025 lb ai/a), Karate Z (lamba-cyhalothrin) at 0.045 kg/ha (0.04 lb ai/a), Baythroid (cyfluthrin) at 0.056 kg/ha (0.05 lb ai/a), Intrepid (methoxyfenozide) at 0.18 kg/ha (0.16 lb ai/a), Steward (indoxacarb) at 0.123 kg/ha (0.11 lb ai/a), Tracer (spinosad) at 0.095 kg/ha (0.085 lb ai/a), Denim (emamectin benzoate) at 0.017 kg/ha (0.015 lb ai/a), and a Mepichlor (mepiquat chloride) at 1.76 l/ha (24 oz/a). The third application was made 30 June at the 12-14 node stage. All treatments remained the same as in the second application, except that Bidrin at a rate of 0.35 kg/ha (0.312 lb ai/a) was added to the tank mix with Mustang Max, Karate Z, and Baythroid. Weed control was visually rated on a scale of 0 to 100% where 0 = no control and 100 = all weeds dead. Crop injury was visually rated on a scale of 0 to 100% where 0 = no crop injury and 100 = total crop injury/all plants dead. Observations were conducted for crop injury on 21 June at 7 days after treatment two (DAT2), and for weed control on 29 June at 15 DAT2. For the third application, crop injury ratings were taken on 7 July at 7 DAT3 and ratings for weed control were taken on 14 July at 14 DAT3. Data were analyzed using Agricultural Research Manager Version 7 using Analysis of Variance and LSD (P=0.10).

**RESULTS AND DISCUSSION**

Results from the ratings after the second application for crop injury indicated that the 0.841 kg/ha (0.75 lb ae/a) rate of MON 3539 showed 13% phytonecrosis at 7 days after treatment two (DAT2) (Table 1). All other treatments ranged from 4 to 20% phytonecrosis. MON 3539 tank mixed with Dimate had the highest rating of 20% phytonecrosis, which significantly differed from that of MON 3539 alone. Several treatments (tank-mixed with MON 3539) showed significantly lower phytonecrosis than MON 3539 alone at 7 DAT2 (Table 1): Bidrin, Trimax, Mustang Max, Karate, Baythroid, Intrepid, Steward, Denim, and Mepichlor. All other treatments did not differ significantly. Weed control in all treatment combinations ranged from 71% to 98% when evaluated 14 DAT2, with MON 3539 having a rating of 84% weed control (Table 1). The only treatment that significantly differed from MON 3539 in weed control at 15 DAT2 was Centric mixed with MON 3539. All other treatments did not significantly differ from that of MON 3539 alone. However, three treatments – Mustang Max, Trimax, and Steward – differed significantly from MON 3539 + Bidrin and MON 3539 + Centric.

Results from evaluations after the third application are indicated MON 3539 had a rating of 5% phyteneosis at 7 DAT3 (Table 2). All other treatments ranged from 5 to 13% phyteneosis. Only the treatment of MON 3539 tank-mixed with Mepichlor had significantly higher phytonecrosis than MON 3539 alone and than MON 3539 tank mixed with Vydate, Baythroid + Bidrin, and Intrepid (Table 2). Weed control in all treatment combinations ranged from 72% to 98% at 14 DAT3, with MON 3539 alone having a rating of 95% control (Table 2). MON 3539 tank mixed with Bidrin, Centric, and Denim showed ratings of 75, 72, and 79% percent control, respectively, which significantly differed from MON 3539 alone and from all other treatments (Table 2).

It should be noted that problems occurred in the tank when mixing certain compounds with MON 3539. Severe flocculation was observed when tank-mixing Trimax with MON 3539. This tank mix was repeated in
the lab with the same general results. A new container of Trimax was used to attempt the tank mix again, causing only minimal flocculation, which was difficult to detect. Finally, the latest experimental formulation of Trimax was used and no flocculation was observed. Settling was observed when Orthene was tank-mixed with MON 3539. When the tank was allowed to remain at rest for more than a few minutes, material in the tank settled to the bottom. This phenomenon was easily corrected by simple agitation. It should be noted that proper, steady agitation may be needed to prevent settling of materials when tank-mixing Orthene with MON 3539.

Certain compounds tank-mixed with MON 3539 in this study showed a significant difference in weed-control effectiveness of MON 3539. MON 3539 tank-mixed with Centric showed a loss of weed control 15 DAT and at 14 DAT. MON 3539 tank-mixed with Bidrin and with Denim showed losses of weed control at 14 DAT. In regard to crop phytonecrosis, Dimate significantly differed from that of MON 3539 alone at 7 DAT, as did Mepichlor at 7 DAT.

ACKNOWLEDGMENTS

The authors would like to thank Chuck Hooker for providing a test location and Monsanto for their support of this study.

LITERATURE CITED


### Table 1. Weed control and phytonecrosis ratings of MON 3539 alone and tank-mixed with selected compounds

<table>
<thead>
<tr>
<th>Treatment and Rate</th>
<th>Rate</th>
<th>Phytonecrosis&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Weed Control&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>MON 3539 (Glyphosate)</td>
<td>0.841 kg/ha (0.75 lb ae/a)</td>
<td>13 b&lt;sup&gt;7&lt;/sup&gt;</td>
<td>84 ab</td>
</tr>
<tr>
<td>MON 3539 + Orthene (Acephate)</td>
<td>1.12 kg/ha (1.0 lb ai/a)</td>
<td>9 bcd</td>
<td>94 ab</td>
</tr>
<tr>
<td>MON 3539 + Bidrin (Dicrotophos)</td>
<td>0.56 kg/ha (0.5 lb ai/a)</td>
<td>6 cd</td>
<td>80 b</td>
</tr>
<tr>
<td>MON 3539 + Vydate C-LV (Oxamyl)</td>
<td>0.529 kg/ha (0.47125 lb ai/a)</td>
<td>11 bc</td>
<td>92 ab</td>
</tr>
<tr>
<td>MON 3539 + Dimate (Dimethoate)</td>
<td>0.56 kg/ha (0.5 lb ai/a)</td>
<td>20 a</td>
<td>94 ab</td>
</tr>
<tr>
<td>MON 3539 + Trimax (Imidacloprid)</td>
<td>0.053 kg/ha (0.0469 lb ai/a)</td>
<td>6 cd</td>
<td>97 a</td>
</tr>
<tr>
<td>MON 3539 + Centric (Thiamethoxam)</td>
<td>0.056 kg/ha (0.05 lb ai/a)</td>
<td>13 b</td>
<td>71 c</td>
</tr>
<tr>
<td>MON 3539 + cypermethrin</td>
<td>0.028 kg/ha (0.025 lb ai/a)</td>
<td>4 d</td>
<td>98 a</td>
</tr>
<tr>
<td>MON 3539 + Karate Z (Lamba-cyhalothrin)</td>
<td>0.045 kg/ha (0.04 lb ai/a)</td>
<td>4 d</td>
<td>90 ab</td>
</tr>
<tr>
<td>MON 3539 + Baythroid (Cyfluthrin)</td>
<td>0.056 kg/ha (0.05 lb ai/a)</td>
<td>4 d</td>
<td>95 ab</td>
</tr>
<tr>
<td>MON 3539 + Intrepid (Methoxyfenozide)</td>
<td>0.18 kg/ha (0.16 lb ai/a)</td>
<td>8 cd</td>
<td>90 ab</td>
</tr>
<tr>
<td>MON 3539 + Steward (Indoxacarb)</td>
<td>0.123 kg/ha (0.11 lb ai/a)</td>
<td>7 cd</td>
<td>97 a</td>
</tr>
<tr>
<td>MON 3539 + Tracer (Spinosad)</td>
<td>0.095 kg/ha (0.085 lb ai/a)</td>
<td>9 bcd</td>
<td>94 ab</td>
</tr>
<tr>
<td>MON 3539 + Denim (Emamectin benzoate)</td>
<td>0.017 kg/ha (0.015 lb ai/a)</td>
<td>6 cd</td>
<td>83 ab</td>
</tr>
<tr>
<td>MON 3539 + Mepichlor (Mepiquat chloride)</td>
<td>1.76 l/ha (24 oz/a)</td>
<td>5 d</td>
<td>89 ab</td>
</tr>
</tbody>
</table>

<sup>1</sup> Application date: 14 June (second application)

<sup>2</sup> Evaluation date: 21 June (7 DAT), 29 July (15 DAT)

<sup>3</sup> Means followed by same letter do not significantly differ (P=0.10, Student-Newman-Keuls).
Table 2. Weed control and phytonecrosis ratings of MON 3539 alone and tank-mixed with selected compounds

<table>
<thead>
<tr>
<th>Treatment and Rate</th>
<th>Rate</th>
<th>Phytonecrosis(\textsuperscript{a})</th>
<th>Weed Control(\textsuperscript{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td>MON 3539 (Glyphosate)</td>
<td>0.841 kg/ha (0.75 lb ae/a)</td>
<td>5 b(\textsuperscript{y})</td>
<td>95 a</td>
</tr>
<tr>
<td>MON 3539 + Orthene (Acephate)</td>
<td>0.841 kg/ha (0.75 lb ae/a)</td>
<td>6 b</td>
<td>96 a</td>
</tr>
<tr>
<td>MON 3539 + Bidrin (Dicrotophos)</td>
<td>0.841 kg/ha (0.75 lb ae/a)</td>
<td>9 ab</td>
<td>75 c</td>
</tr>
<tr>
<td>MON 3539 + Vydate C-LV (Oxamyl)</td>
<td>0.529 kg/ha (0.47125 lb ai/a)</td>
<td>5 b</td>
<td>91 a</td>
</tr>
<tr>
<td>MON 3539 + Dimate (Dimethoate)</td>
<td>0.56 kg/ha (0.5 lb ai/a)</td>
<td>10 ab</td>
<td>96 a</td>
</tr>
<tr>
<td>MON 3539 + Trimax (Imidacloprid)</td>
<td>0.053 kg/ha (0.0469 lb ai/a)</td>
<td>6 b</td>
<td>90 ab</td>
</tr>
<tr>
<td>MON 3539 + Centric (Thiamethoxam)</td>
<td>0.056 kg/ha (0.05 lb ai/a)</td>
<td>6 b</td>
<td>72 c</td>
</tr>
<tr>
<td>MON 3539 + cypermethrin + Bidrin (Dicrotophos)</td>
<td>0.841 kg/ha (0.75 lb ae/a)</td>
<td>6 b</td>
<td>98 a</td>
</tr>
<tr>
<td>MON 3539 + cyhalothrin + Bidrin (Dicrotophos)</td>
<td>0.841 kg/ha (0.75 lb ae/a)</td>
<td>6 b</td>
<td>85 ab</td>
</tr>
<tr>
<td>MON 3539 + Baythroid (Cyfluthrin) + Bidrin (Dicrotophos)</td>
<td>0.841 kg/ha (0.75 lb ae/a)</td>
<td>5 b</td>
<td>89 ab</td>
</tr>
<tr>
<td>MON 3539 + Intrepid (Methoxyfenozide)</td>
<td>0.18 kg/ha (0.16 lb ai/a)</td>
<td>5 b</td>
<td>95 a</td>
</tr>
<tr>
<td>MON 3539 + Steward (Indoxacarb)</td>
<td>0.123 kg/ha (0.11 lb ai/a)</td>
<td>8 b</td>
<td>97 a</td>
</tr>
<tr>
<td>MON 3539 + Tracer (Spinosad)</td>
<td>0.095 kg/ha (0.085 lb ai/a)</td>
<td>8 b</td>
<td>97 a</td>
</tr>
<tr>
<td>MON 3539 + Denim (Emamectin benzoate)</td>
<td>0.017 kg/ha (0.015 lb ai/a)</td>
<td>9 ab</td>
<td>79 bc</td>
</tr>
<tr>
<td>MON 3539 + Mepichlor (Mepiquat chloride)</td>
<td>1.76 l/ha (24 oz/a)</td>
<td>13 a</td>
<td>86 ab</td>
</tr>
</tbody>
</table>

\(\textsuperscript{a}\)Application Date: 30 June (Third Application)
Evaluation Date: 7 July (7 DAT), 14 July (14 DAT)
\(\textsuperscript{y}\)Means followed by same letter do not significantly differ (P=0.10, Student-Newman-Keuls).
Fig. 1. Comparison of phytonecrosis ratings after treatments 2 & 3

Fig. 2. Comparison of weed control ratings after treatments 2 & 3