

Effect of Decortivating Sorghum on Ethanol Production and Composition of DDGS¹

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ABSTRACT

Cereal Chem. 83(1):17–21

The use of a renewable biomass that contains considerable amounts of starch and cellulose could provide a sugar platform for the production of numerous bioproducts. Pretreatment technologies have been developed to increase the bioconversion rate for both starch and cellulosic-based biomass. This study investigated the effect of decortication as a pretreatment method on ethanol production from sorghum, as well as investigating its impact on quality of distillers' dry grains with solubles (DDGS). Eight sorghum hybrids with 0, 10, and 20% of their outer layers removed were

used as raw materials for ethanol production. The decorticated samples were fermented to ethanol using *Saccharomyces cerevisiae*. Removal of germ and fiber before fermentation allowed for greater starch loading for ethanol fermentation and resulted in increased ethanol production. Ethanol yields increased as the percentage of decortication increased. The decortication process resulted in DDGS with higher protein content and lower fiber content, which may improve the feed quality.

During the last 20 years, many industries and manufacturers have been seeking to replace petroleum-based feedstocks with renewable materials. Use of renewable biomass, which contains significant amounts of starch or cellulose, could provide a sugar platform for numerous bioproducts. Ethanol demand is growing as a "clean" substitute for direct use as fuel, which can ease both natural resource limitation and environmental pollution (Roehr 2001). U.S. ethanol production capacity is projected to increase from 3.1 billion gallons in 2003 to 6.0 billion gallons per year by 2006 (MacDonald et al 2003).

Starch-rich grains such as maize and sorghum are viable renewable resources for ethanol production (Turhollow and Heady 1986; Christakopoulos et al 1993; Lezinou et al 1995; Dien et al 2002). Grain sorghum (*Sorghum bicolor* (L) Moench) is one of the most important crops in the United States, and its production ranks third among cereal crops. Sorghum contains 55–75% starch by kernel weight (Serna-Saldivar and Rooney 1995). Sorghum is a tropical grass grown primarily in semiarid and dry parts of the world, especially in areas too dry for maize. The diversity of climate that sorghum can grow in, as well as the fact that it is heat- and drought-tolerant, makes sorghum an important cereal crop, especially in arid areas of the world. About 90% of U.S. sorghum production currently is used for animal feed and only ≈10% for ethanol production.

Pretreatment technologies have been developed to increase the conversion rate of biomass, including mechanical methods such as size reduction through milling, decortication, and the extrusion processes; physical methods such as steaming, radiation, and sonication; chemical methods such as alkaline and acid hydrolysis; biological methods such as microbial and enzyme degradation; and a combination of these methods.

Decortication is the removal of the bran or outer layers of the grain. Abrasive decortication operates on the principle of progressively rubbing off the outer layers of the kernel (Beta et al 2000). MacLean et al (1983) studied the effect of decortication on the

apparent protein quality and digestibility of sorghum. They suggested that the use of decortication can markedly improve the apparent protein quality and digestibility of sorghum. Higirot et al (2003) compared the quality and yield of starch between sorghum grits from milling techniques using roller mills and grits from a decorticator-degerminator. The grits obtained from the decorticator-degerminator had greater starch recovery (61–70%) than did the grits from roller milling (51–68%). Beta et al (2000) also demonstrated that abrasive decortication and roller milling reduced the levels of polyphenols from high-tannin sorghum. Tannin is the primary nutrient-limiting component in sorghum lines with a pigmented testa. High concentrations of tannin may have a negative effect on the fermentation process, resulting in as much as a 10% reduction of starch and protein digestibility (Leeson and Summers 1997). For those reasons, decortication may increase the bioconversion rate of sorghum in sorghum lines containing tannins (i.e., those with a pigmented testa). Decortication may also reduce other fermentation inhibitors such as phenolic acid, color compounds, etc.

Ponnampalam et al (2004) studied the effect of germ and fiber removal on ethanol production from maize. They reported that the integration of germ and fiber removal in the dry-grind ethanol industry could increase fermentation capacity and add value to co-products, resulting in increased productivity and profits. Decortication not only increases the starch loading for fermentation but also changes the chemical composition of distillers' dried grains with solubles (DDGS). DDGS is a major co-product from ethanol production. Rasco et al (1987) reported that the protein content increased 2.4 to 3.1 times, crude fiber increased 2.6 to 3.8 times, lipid increased 1.4 to 2.4 times, and ash increased 3.8 to 7.8 times for DDGS from maize and wheat, compared with the corresponding starting raw material. As more ethanol processing plants are built in response to demand for fuel ethanol, there will be an increasing supply of DDGS. Dry-mill ethanol plants currently produce more than 5.5 million metric tons of DDGS annually. Industry experts predicted that the volume of DDGS produced would be over 7.5 million metric tons by the end of 2005 (Kansas Sorghum Commission, Paola, KS). Most of DDGS are currently used for animal feeds. By finding more uses of DDGS and improving DDGS quality, ethanol plants can potentially maintain or improve their profitability, even as competition increases (Davis 2001). Therefore, marketing of DDGS is critical to the economic stability of dry-grind ethanol plants (Renewable Fuels Association 2005). Belyea et al (2004) studied chemical composition of DDGS. They reported that DDGS are composed mainly of protein, fat, crude fiber, and starch. Protein is the most important nutrient in animal feed; protein variation in feeds can cause misformulation and can affect animal productivity. Rai et al

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³ USDA-ARS Grain Marketing and Production Research Center, Manhattan, KS 66502. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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(2004) reported that the DDGS from maize contains 27–35% protein and 8–12% fat. For grain sorghum, DDGS contains ≈30.3% protein, 12.5% fat, and 10.7% crude fiber (Kansas Sorghum Commission). Both fat and protein affect market value; DDGS with high protein (>30%) and high fat (>12%) is worth about \$5–\$20 per ton more than DDGS with lower protein (<28%) and lower fat (<11%) (Belyea et al 2004).

Decortication ideally should separate all bran with attached wax and minimal starch from sorghum kernels. Removal of the bran before fermentation is expected to increase the digestibility of sorghum starch and to increase the starch load and fermentation efficiency. Therefore, the objective of this research was to study the effect of the decortication process as a pretreatment method for ethanol production and DDGS quality.

MATERIALS AND METHODS

Decortication Process

Eight sorghum hybrids were decorticated using a tangential abrasive dehulling device (TADD) equipped with an 80-grit abrasive pad (Venebles Machine Works, Canada). The abrasive pad was shimmed to minimum distance from the upper plate. Decorticated grains were collected for ethanol fermentation. Because bran was not used for ethanol fermentation in this research, the chemical composition of bran was not analyzed. The decorticated samples were milled (cyclone sample mill, Udy Corp., Fort Collins, CO) into powder with a particle size of <2 mm and were used as a substrate for ethanol fermentation. The moisture content of these samples was determined using Approved Method 44-15A (AACC International 2000).

Microorganisms

Saccharomyces cerevisiae (ATCC 24860) was used for ethanol fermentation. Yeast cells were maintained on YPD medium with 20 g of yeast extract/L, 5 g of peptone/L, 5 g of dextrose/L, and 20 g of agar/L. Yeast cells were cultured in a rotatory shaker with a shaker speed of 200 rpm at 30°C for 48 hr in a preculture media (2% glucose, 0.5% peptone, 0.3% yeast extract, 0.1% KH₂PO₄, and 0.05% MgSO₄ · 7H₂O at pH 5.5).

Ethanol Fermentation

Termamyl 120 L (0.01 mL of α-amylase/g of dry starch) (Novozymes North America, Franklinton, NC) was used for starch liquefaction. Erlenmeyer flasks (250 mL) with a 100-mL medium

containing 200 g of starch substrate/L, 3 g of peptone/L, 1 g of KH₂PO₄/L and 1 g of (NH₄)₂SO₄/L at pH 5.8 were placed in the temperature-controlled water bath shaker at 95°C with an agitation speed of 140 rpm (model Giramax 939 XL). After lowering the temperature to 80°C, the second Termamyl 120 L (0.01 mL of α-amylase/g of dry starch) was added and the liquefaction was continued for 30 min with continuous agitation at 140 rpm. Amyloglucosidase solution (3,000 U/mL) was used for starch saccharification based on 150 U/g of dry starch at 60°C with continuous agitation in the water bath shaker for 30 min at 140 rpm.

After saccharification, the fermentation medium was adjusted to pH 3.85. The medium was then inoculated with 6% yeast suspension (1 × 10⁶ cells/mL) and incubated in a rotatory shaker (200 rpm) at 30°C for 72 hr. All experiments were duplicated and the average values were reported.

After completion of the distillation, the whole broth was dried at 49°C until the moisture content was <15% (wb). A coffee grinder was used to homogenize the dried samples so they represented the distillers' dried grains with solubles (DDGS).

Analysis Methods

Starch content was determined using commercially available kits from Megazyme (Bray, Ireland) and Approved Method 76-13 (AACC International 2000). Protein was determined by nitrogen combustion using a nitrogen determinator (LECO FP-528, St. Joseph, MI) according to Approved Method 46-30 for crude protein-combustion. Nitrogen values were converted to protein content as N × 6.25. Crude fiber, fat, and ash were determined using AOAC standard methods (AOAC International 1995).

Free sugar was measured as glucose before and after fermentation using the Lane and Enyon volumetric method (Plews 1970). Calcium and phosphorous were determined according to AOAC standard method 968.08. Ethanol was obtained by distillation of fermentation broth. Ethanol concentration was determined according to the specific-gravity method 942.06 (AOAC International 1995). All experiments were duplicated and the average values were reported.

Kernel colors (Hunter *L*, *a*, and *b* values) of the undecorticated and decorticated sorghum were obtained using a colorimeter (Hunter Color Quest 45/0). In *L-a-b* color space, *L* varies from 0 (black) to 100 (perfect white); *a* measures green when negative and red when positive; and *b* is a measure of blue when negative and yellow when positive.

TABLE I
Chemical Composition of Sorghum as Affected by Degree of Decortication

	Chemical Composition (%)						Chemical Composition Change (%)	
	0% D ^a		10% D		20% D		0% vs. 10% D	0% vs. 20% D
	Average	Range	Average	Range	Average	Range		
Starch	73.93	69.3–76.6	78.7	73.5–81.4	82.9	80.1–85.8	4.9–7.1	8.6–15.6
Protein	10.48	9.7–11.1	10.21	9.4–11.2	9.64	8.6–11.0	–(9.0–1.4) ^b	–(12.0–4.8)
Crude fiber	1.53	1.3–1.8	0.64	0.49–0.81	0.25	0.14–0.41	–(62.7–49.2)	–(89.4–74.4)
Crude fat	3.57	3.0–4.1	3.04	2.5–3.4	2.37	2.1–2.7	–(20.5–12.0)	–(38.6–28.9)

^a D, decortication percentage.

^b KS11 and KS15 were not included.

TABLE II
Color Variations of Sorghum Flours as Affected by Degree of Decortication

Sorghum Samples	<i>L</i> Value		<i>a</i> Value		<i>b</i> Value	
	Average	Range	Average	Range	Average	Range
0% D ^a	63.0	56.9–70.9	8.94	2.5–11.8	19.1	16.1–21.5
10% D	70.4	65.1–77.6	6.93	1.2–11.1	16.9	15.4–18.6
20% D	75.3	68.6–81.1	5.53	0.5–10.6	15.1	13.4–17.2

^a D, decortication percentage.

Analysis of variance (ANOVA) and least significant difference (LSD) were determined using the Statistical Analysis System (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Effect of Decortication on Chemical Composition and Kernel Color of Sorghum

The initial chemical composition ranges of sorghum samples were 69.3–76.6% for starch, 9.7–11.1% for protein, 3.0–4.1% for crude fat, and 1.3–1.8% for crude fiber (Table I). Decortication had a significant effect on the chemical composition of the decorticated kernels. Starch content increased significantly as the degree of decortication increased, whereas the crude fiber and crude fat contents decreased significantly as the degree of decortication increased (Table I). This occurs because the decortication process removes the outer layers of the kernels, including the pericarp and germ, which are higher in protein, fiber, and fat, and lower in starch than the endosperm.

In general, protein content decreased as the degree of decortication increased, except in samples KS11 and KS15 with 1.0% and 0.9% increase (data not shown). The exceptions may have been due to the differences in the thickness of the pericarp in those samples or in the distribution of the protein within the endosperm. The starch content was increased by 4.9–7.1% for sorghums with 10% decortication and by 8.6–15.6% for sorghums with 20% decortication, respectively; the crude fat content was decreased by 12.0–20.5% and by 28.9–38.6% for sorghums with 10% and 20% decortication, respectively; and crude fiber content was decreased by 49.2–63.4% and by 74.4–89.4% for sorghums with 10% and 20% decortication, respectively (Table I). Crude protein content was decreased by 1.4–9.0% and by 4.9–12.0% for 10% and 20% decortifications, respectively, except in samples KS11 and KS15. These changes in composition allow for greater starch loading during fermentation, which benefits the fermentation process by increasing the fermentable substrate and fermentation yields. For example, with the same substrate concentration, the starch loading would increase 5–15% when the decorticated sorghum was used.

Decortication had a significant effect on grain color. The percentage of luminance increased as the degree of decortication increased. Thus, the *L* values of the kernels increased significantly as the degree of decortication increased, which was expected (Table II). This indicates that whiteness of the grain increased as percentage of decortication increased. On the other hand, *a* values decreased as the degree of decortication increased. The *b* values also decreased as the degree of decortication increased, indicating that the sorghum samples were less yellow after decortication. These changes suggest that DDGS color would change with decorticated sorghum. Lighter colored DDGS may avoid the problem of the DDGS seeming burnt during the drying process, which is often mistaken for lower quality DDGS.

Ethanol Production

Ethanol yields increased significantly as the degree of decortication increased. Removal of hull and outer layer pericarp before fermentation optimized starch digestion and increased ethanol production. As previously mentioned, decortication resulted in samples with greater starch contents and increased the amount of fermentable substrate in turn, resulting in increased fermentation yields. Decortication may have other benefits for fermentation including the removal of fermentation inhibitors such as phenolic acids, polyphenols (when present), and color compounds present in the bran. Decortication may also increase the access of the endosperm to the enzymes used during the fermentation process by removing bran that could be attached to endosperm particles during milling. Research is in progress to determine whether decortication increases the rate of ethanol fermentation for this reason.

Ethanol yield ranges were 8.2–9.1% for undecorticated samples, 9.0–9.7% for sorghums with 10% decortication, and 9.3–10.2% for sorghums with 20% decortication when 20% substrate concentration was used (Table III). The ethanol yields increased 3.3–11.1% for sorghums with 10% decortication and increased 7.6–18.1% for sorghums with 20% decortication. The samples with the maximum increase in the percentage of starch due to the decortication were KS1, KS2, and KS15 with 20% decortication. The greatest increase in starch resulted in the greatest ethanol

TABLE III
Ethanol Yields as Affected by Degree of Decortication

Sample	Ethanol Yields (% v/v)			Ethanol Increase (%)	
	0% D ^a	10% D	20% D	0% vs. 10% D	0% vs. 20% D
KS 1	8.19a ^b	9.10b	9.49c	11.11	15.87
KS 2	8.62a	9.56b	10.18c	10.90	18.10
KS 3	9.08a	9.37b	9.77c	3.26	7.58
KS 4	9.03a	9.69b	10.04c	7.26	11.16
KS 11	8.75a	9.16b	9.76c	4.71	11.62
KS 15	8.29a	8.99b	9.25c	8.42	11.60
KS 20	8.93a	9.54b	9.81c	6.83	9.85
KS 23	8.85a	9.47b	9.72c	7.01	9.83

^a D, decortication percentage.

^b Values followed by the same letter in the same row are not significantly different ($P < 0.05$).

TABLE IV
Ethanol Concentration (%) as a Function of Substrate Content and Degree of Decortication^a

Sample Substrate (%)	Ethanol Yields (% v/v)			Ethanol Increase (%)	
	0% D ^b	10% D	20% D	0% vs. 10% D	0% vs. 20% D
20	8.41a ^c	9.33b	9.84c	11.01	16.98
25	10.69a	11.79b	12.21c	10.29	14.22
30	13.23a	14.53b	14.98c	9.83	13.23
35	15.42a	16.85b	17.30c	9.27	12.19

^a Data reported are based on ethanol yields from KS1 and KS2.

^b D, decortication percentage.

^c Values followed by the same letter in the same row are not significantly different ($P < 0.05$).

yields. The percentage increases in ethanol yields for these samples were 15.9% KS1, 18.1% KS2, and 11.6 % KS15 (Table III).

Results indicated that starch loading is the major factor affecting ethanol yield. To further study the effect of substrate concentration on ethanol fermentation and quality of DDGS, KS1 and KS2 were selected, and substrate concentrations of 20, 25, 30, and 35% were used. In general, ethanol yields increased as substrate concentration and degree of decortication increased (Table IV). The incremental change in the ethanol yield, expressed as a percentage is also shown in Table IV. Although the increment increased with increasing decortication, it decreased as the substrate loading was elevated. This may be because fermentation achieved an ethanol concentration that inhibited further ethanol fermentation by yeast. Ethanol concentration is one of three major factors (temperature, acidity, and ethanol concentration) affecting ethanol yield; the rising concentration of ethanol due to the increase in the substrate concentration and greater initial starch loads resulting from decortication tended to have an inhibitory effect on the fermentation process. This effect is partly due to feedback inhibition whereby accumulation of the end products of a process tends to slow the process itself. With 10% decortication, the percentage of ethanol increment increased by 9.3–11.0%. With 20% decortication, the ethanol increased by 12.2–17.0%.

Chemical Composition of DDGS

Protein content is the most important quality factor for marketing and end-use quality of DDGS (Belyea et al 2004). DDGS

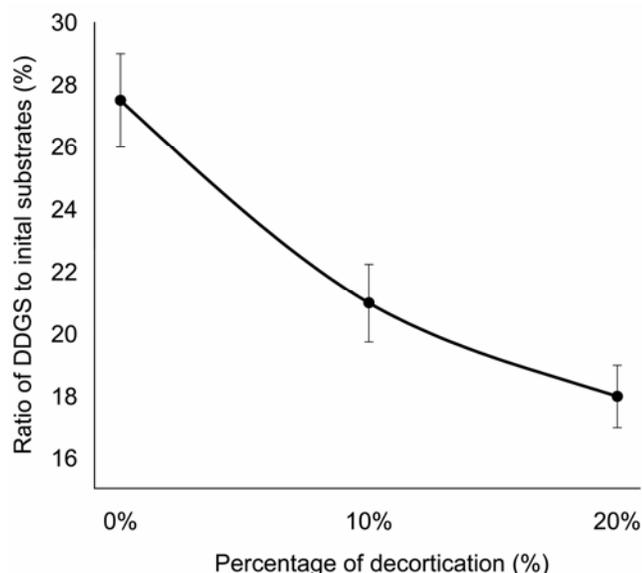


Fig. 1. Effect of decortication on the ratio of DDGS to initial substrate.

from decorticated sorghum contains much more protein than does the original sorghum. Protein content of DDGS increased significantly as the degree of decortication increased (Table V). Protein content of DDGS ranges were 39.9–45.1% for undecorticated sorghums, 46.6–53.1% for sorghums with 10% decortication, and 51.6–56.8% for sorghums with 20% decortication. The ranges of percentage increase of protein content in DDGS were 11.1–25.6% for sorghums with 10% decortication and 20.8–38.9% for sorghums with 20% decortication. The increase of protein content may increase the market value of DDGS and have an impact on its feed quality. The increase in protein content of DDGS could be caused by two main factors: yeast growth and sorghum composition. As yeast grows during fermentation, cell mass as yeast protein increased significantly, and this contributes to the final protein content of DDGS. Decortication slightly decreased protein content as a mass portion in the decorticated kernels but significantly increased starch content up to 9% (Table I). This means that decortication can decrease the total DDGS yield as much as 12%. With a small percentage of protein decrease and a large percentage of starch increase, the protein content of DDGS must increase significantly.

Because starch in sorghum is converted to ethanol and removed, the remaining nutrients in the grain are concentrated and often increased two- or threefold in the resultant DDGS. Compared with the original samples, the most important changes were a five- to sixfold increase in protein concentration, a five- to sixfold increase in fat content, a 12 to 14-fold increase in crude fiber content, and a 15-fold decrease in starch when sorghums with 20% decortication were used. Although the starch content in DDGS was similar and the ratio of DDGS to initial substrate decreased as the degree of decortication increased, the amount of unfermented starch decreased as the degree of decortication increased (Fig. 1). The smaller amount of starch in DDGS resulting from decorticated samples, compared with the DDGS from the original grain, indicates that decortication increased the starch conversion rate. The decortication process also reduced the crude fiber and increased the starch and crude oil contents of DDGS, which must increase the feeding value and energy content of DDGS (Table V). Although most of the attention is given to the amount of protein in DDGS, fat and starch are also important nutrients because they increase available energy concentrations (Belyea et al 2004). Phosphorous, calcium, and ash were not severely affected by the degree of decortication. This is significant because their presence is important as valuable nutrients for animal feeds.

Most (≈98%) of DDGS in North America currently comes from both maize and sorghum ethanol fermentation (Belyea 2004). The remaining 1–2% of DDGS is produced by the alcohol beverage industry. Approximately 5.5 million metric tons of DDGS are produced annually in the United State. It would be beneficial to compare the nutritional values of DDGS from sorghum with other

TABLE V
Chemical Composition (%) of DDGS (% DM) of Different Grains

	Protein	Starch	Fiber	Fat	Ash	Phosphorous	Calcium
Decorticated sorghum							
0% D ^a	39.9–45.1	5.2–5.2	7.5–9.2	10.8–12.0	3.6–3.8	0.81–0.85	0.03–0.04
10% D	46.6–53.1	5.4–5.5	5.8–6.9	11.4–13.0	3.6–4.0	0.85–0.86	0.03
20% D	51.6–56.8	5.6–5.7	3.4–4.7	11.8–14.2	3.9–3.9	0.79–0.81	0.03
Wheat ^{b,c}	19.6–35.6	–	5.6–7.6	3.9–7.7	7.4–9.4	0.96	0.15
Corn ^{b,d}	23–31.3	5.1	6.3–10.2	9.0–11.9	4.6–12.1	0.84	–
Sorghum ^{e,f}	30.3–45.3	5.7	10.7–11.6	12.3–12.5	2.1–5.3	0.84	0.10

^a D, decortication percentage.

^b Rasco et al (1987).

^c Mohawk Canada, Ltd.

^d Belyea et al (2004).

^e Wu et al (1984).

^f SoueHigh Plains Corporation, Colwich, KS.

grains. Some published results related to chemical composition of DDGS from different grains are also summarized in Table V. Protein contents of DDGS from both undecorticated and decorticated sorghum (≈ 39.9 – 45.1% for undecorticated sorghum, 46.6 – 53.1% for 10% decorticated sorghum, and 51.6 – 56.8% for 20% decorticated sorghum) were much greater than the protein in DDGS from corn and wheat (23 – 31.3% for corn; 19.6 – 35.6% for wheat). Although likely fermented under different conditions with different amounts of added yeast, nutrients, etc., the decorticated sorghum samples used in this study had much higher protein contents than the other grains listed in Table V. Fat content was also greater than that in wheat and corn. Ash, starch, and phosphorous were well within the ranges reported by Wu et al (1984) and the High Plains Corporation (Wichita, KS). The ash content is lower than that of wheat and corn, suggesting there was no significant effect of salt formation during pH adjustments before fermentation.

CONCLUSIONS

Removal of the outer layer of the pericarp before fermentation by the decortication process allowed greater starch loading and resulted in increased ethanol yields. Ethanol yield increased as decortication and substrate concentration increased. The ethanol yields increased by 3.3 – 11.1% for sorghums with 10% decortication and by 7.6 – 18.1% for sorghums with 20% decortication when 20% substrate content was used. The ethanol yields increased 9.3 – 14.2% when the substrate concentrations were higher than 20% . Decortication also resulted in significant changes in the chemical composition of DDGS. Protein and fat contents of DDGS increased and crude fiber decreased as the degree of decortication increased. Decortication had no significant effect on starch, ash, calcium, and phosphorous contents in DDGS. The protein content of DDGS from sorghum was greater than that reported for maize and wheat, which may result in sorghum DDGS having greater feed quality.

ACKNOWLEDGMENTS

This project was supported in part by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2004-35504-14808 and Specific Cooperative Research Agreement No. 58-5430-3-309 with the Grain Marketing and Production Research Center, Agricultural Research Services, U.S. Department of Agriculture.

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[Received March 29, 2005. Accepted August 23, 2005.]