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Development and fecundity rate of *Tribolium castaneum* (Herbst) on Distillers Dried Grains with Solubles

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ABSTRACT

This research focused on the influence of two samples of corn Distillers Dried Grains with Solubles (DDGS) obtained from an "old" generation dry-grind fuel ethanol plant as a food and oviposition resource for red flour beetle, *Tribolium castaneum*, in contrast with traditional flour (90%)/yeast (10%) diet. Larval development was significantly faster on a flour/yeast diet (18.6 d) compared to the DDGS sample 1 (44.1 d) and DDGS sample 2 (34.5 d). DDGS sample 1 had the highest larval mortality (38.7%) with a wider mortality range (6.7–66.7%) compared with flour/yeast (4.4%, range 0–14.3%) and DDGS sample 2 (7.1, range 0–26.7%). Both DDGS diets and the flour/yeast diet had no significant influence on egg incubation period or pupation time and percentage of egg hatching or pupal mortality. Additionally, fecundity was significantly lower on DDGS compared to the flour/yeast diet (18.0, 36.5, and 175.5 eggs per female on DDGS sample 1, DDGS sample 2, and flour/yeast diet, respectively). These results indicate that this type of DDGS is not a suitable developmental diet compared to the standard laboratory diet and that the addition of this type of DDGS to animal feeds should not increase feed vulnerability to flour beetle infestation. A comparison of DDGS samples by particle size indicated that the larger particle size, sample 1 was less suitable for *T. castaneum* oviposition and development.

1. Introduction

Distillers Dried Grains with Solubles (DDGS), a co-product produced from converting corn into fuel ethanol using the dry-grind process, is used in livestock feeds to replace grains as an ingredient. The majority of DDGS produced in the U.S. is from No. 2 yellow shelled corn. In 2010, 116.2 million tons of corn (4.65 billion bu) was converted into about 49.2 billion liters (13 billion gallons) of fuel ethanol and 32.5 million metric tons of high value feed (RFA, 2011a). Most of the high value feed co-products, about 30 million metric tons, that is produced from fuel ethanol production is distillers grains, and about 18.3 million metric tons (61%) dried to DDGS (RFA, 2011a). DDGS is produced by blending and drying the non-fermentable residues in corn after fermentation of the starch, i.e., distillers wet grains (DWG) and condensed distillers solubles (CDS) (Kingsly et al., 2010).

After starch is fermented into ethanol, the remaining unfermentable solids which make up DDGS contains protein, fiber, oil, and ash with approximately 2–3 times higher concentration than in the raw grain (corn) (Shurson et al., 2003). Studies show that DDGS from different plants vary in color, odor, concentration of nutritional elements and digestibility (Cromwell et al., 1993), as well as particle size and bulk density (Shurson et al., 2003). This variation can affect the growth rate and weight gain of chicks and pigs (Cromwell et al., 1993).

DDGS have been used in animal feeds, especially pigs and cattle, or finishing diets in various types of feed production, to replace corn or other grains (Shurson et al., 2003; Stein and Shurson, 2009). DDGS is generally priced 10–25% lower than corn which has increased the market demand for DDGS as a feed ingredient (RFA, 2011b). As more of this product is available to the feed industry, understanding the effect it might have on the vulnerability of animal feed to insect infestation is necessary. Knowledge of insect biology on a diet is vital for safe storage of that diet. There are no published studies that have investigated the susceptibility of DDGS as a raw ingredient to insect infestation. Thus, the objective of this study was to examine the vulnerability of DDGS obtained from an "old" generation dry-grind process plant to infestation by red flour beetle (*Tribolium castaneum* (Herbst)), by determining its development and fecundity rate on DDGS in contrast with a standard laboratory diet.

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2. Materials and methods

2.1. Insects and diets

Tribolium castaneum colonies were maintained in series I-36 controlled environmental chambers at 27 ± 1 °C in the Department of Entomology at Purdue University, West Lafayette, IN USA. A diet of wheat flour (90%) and brewer's yeast (10%) was used for colony maintenance and as the control diet for comparison with two samples of DDGS products. DDGS samples used in this experiment were obtained from an "old" generation dry-grind fuel ethanol process plant and varied in particle size. An "old" generation plant is defined as one constructed in the early 1980s compared to "new" generation dry-grind fuel ethanol process plant since built after 1990 (Ileleji et al., 2007).

The DDGS samples were collected after particle segregation of the DDGS into a conical pile by gravity-driven-discharge without vibration from a reservoir with a funnel-shaped bottom mounted on a wooden platform. Then, the DDGS conical pile was divided into four portions by using three steel rings from the center toward the periphery forming concentric circles from the peak. Portions were numbered 1–4 from the center to its periphery and they were labeled inner core, outer core, inner periphery and outer periphery, respectively according to Ileleji et al. (2007). The two samples of DDGS used in this study were a well mixed sample of all four portions of the pile (DDGS sample 1) and a sample from the second circle (outer core) of the pile (DDGS sample 2). DDGS particle size as measured by the geometric mean diameter (d_{wg}) for four segregated portions of the well mixed sample, DDGS sample 1 ranged from 793.4 to 1327.3 μm, while the d_{wg} for the outer core of the segregated sample during gravity-driven discharge, DDGS sample 2, was 829.4 μm (Ileleji et al., 2007). Diets were kept refrigerated for longer preservation and DDGS diets were re-blended by stirring with a large plastic spoon before using in experiments to mix segregated particles.

2.2. Developmental rate

Tribolium castaneum eggs were obtained by placing about 100 adult beetles on a thin layer of wheat flour (90%) and brewers' yeast (10%) in a jar (400 ml) for 24 h at 32.5 ± 1 °C in the environmental chamber. One-day-old eggs were sifted from the diet using a No. 80 sieve (Seedburo Equipment Company (Des Plaines, IL, USA)/0.18 mm hole size). Under microscope and using a small paint brush with most of the bristles removed, eggs were placed singly in the wells of a 16-well plate filled with 0.125 ml of one of the diets so that the eggs were in contact with the diet and could be seen easily to determine the egg incubation period. Wells were checked twice a day until larval emergence. Once larvae emerged, cells where half filled (~1 ml) with the same diet and for the duration of larval and pupal stages. After 12 d of feeding, late instars larvae in well plates were checked daily until adult emergence. A total of 18 16-well plates were used for the flour/yeast diet, 17 16-well plates for DDGS sample 1 and 17 16 well-plates for DDGS sample 2.

2.3. Fecundity

Pupae from a laboratory colony were sexed using external genital characteristics (Halstead, 1963; Shukla and Palli, 2012) and kept in separate containers until adult emergence. One pair of 3–5 d-old adults was placed in a Petri dish half-filled (20 ml) with either test diet and held at 32.5 ± 1 °C in the environmental chamber. Preliminary experiments indicated the unsuitability of DDGS sample 1, thus more DDGS sample 1 dishes were prepared (sixty two dishes of flour/yeast diet and seventy two dishes of DDGS sample 1 and thirty four dishes of DDGS sample 2). After a 3 wks oviposition period, both adults were removed from the Petri dish. Since it was difficult to separate eggs from the DDGS diet, the numbers of larvae alive after two additional weeks were counted. Since developmental growth was faster on flour/yeast diet, we started to check the flour/yeast Petri dishes at the end of the 4th week and only removed insects when they pupated. Thus fecundity numbers recorded reflect the number of eggs laid, less those eggs that did not hatch and those that did not survive the first two weeks of life. In order to separate the larvae in flour/yeast a No. 80 sieve (Seedburo Equipment Company (Des Plaines, IL, USA)/0.18 mm hole size) was used while for the DDGS diets a No. 80 sieve, No. 18 sieve (Seedburo Equipment Company (Des Plaines, IL, USA)/1.0 mm hole size) and No. 14 sieve (Seedburo Equipment Company (Des Plaines, IL, USA)/1.4 mm hole size) were used to separate particle size. The content of each sieve was placed on a piece of paper and examined to determine the number of larvae.

2.4. Data analysis

In each well plate, the average number of days for each life stage was determined and then these were compared by diet (F/Y, DDGS samples 1 and 2) and life stage. Numbers were not uniform since some life stages died and did not transfer to the next stage. Due to non-normality, data were log transformed before statistical analysis. A one-way ANOVA was used for comparing mortality and egg hatch percentage, while one-way ANOVA and Tukey HSD test were used to compare average number of days to complete each life stage for the three treatments. Data were run on SAS version 9.2 (SAS Institute, 2008). Standard errors of means are reported.

3. Results and discussion

3.1. Developmental rate

Larval period, in contrast with other stages of development, significantly increased when fed on a diet of DDGS (Table 1) while

Table 1
Developmental period (±SE), egg hatch, and larval and pupal mortality rates of *Tribolium castaneum* fed three different diets with comparable published data.

Life stage	Flour/yeast	DDGS sample 1	DDGS sample 2	Wheat feed (Howe, 1956)	*F* (ANOVA)	*P*
Egg						
Development (d)	3.8 ± 0.01a	3.8 ± 0.02a	3.9 ± 0.01a	2.9	$1.7_{(2,49)}$	0.2
Hatch (%)	94.8a	94.1a	91.9a	75	$1.3_{(2,49)}$	0.3
Larvae						
Development (d)	18.6 ± 0.3a	44.1 ± 1.0c	34.5 ± 0.4b	14.6	$569.8_{(2,49)}$	<0.01
Mortality (%)	4.4a	38.7b	7.9a	7	$50.9_{(2,49)}$	<0.01
Pupal						
Development (d)	4.8 ± 0.1a	4.8 ± 0.0a	4.8 ± 0.1a	4.6	$0.02_{(2,49)}$	0.98
Mortality (%)	4.9a	2.6a	1.3a	2.5	$2.9_{(2,49)}$	0.1

Means followed by the same letter in each column are not significantly different (Tukey's test, $P > 0.05$).