Journal of Stored Products Research 87 (2020) 101606

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Development of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) on sorghum milling fractions

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ARTICLE INFO

Article history:
Received 10 February 2020
Received in revised form
23 March 2020
Accepted 25 March 2020
Available online 22 April 2020

Keywords:
Sorghum
Fractions
Insects

ABSTRACT

A series of tests was conducted to determine if *Tribolium castaneum* (Herbst), the red flour beetle, could survive on six milled sorghum fractions: Bran, Coarse Grits, Fine Grits, Flour, Red Dogs, and Shorts. In the first test, parental adults were exposed on the fractions, removed, and then the fractions were held for six-seven weeks at 27 °C. Late instar larvae and progeny adults were present in all fractions. In the second test, at least 80% of single neonates (1-2-day-old larvae) held on 1 g of a fraction at 27 °C for seven weeks were able to complete development to the adult stage. In the final test, individual neonates were held on 1 g of a fraction at 37 °C, 32 °C, 27 °C, or 22 °C. Time to adult emergence at each temperature ranged from 17 to 23 days, 21–27 days, 28–50 days, and 67–113 days, respectively, depending on the specific fraction. Logistic functions were compared for mean developmental times for each temperature-fraction combination. The six fractions were also analyzed for ash, fat, fiber, moisture, protein, and starch content. The fractions varied with respect to these chemical constituents; fat and moisture content were negatively correlated with development in some comparisons, though overall there was no correlation between these chemical components and neonate development on the fractions. Temperature had an obvious effect on neonate development, which has implications for assessing risk of pest infestations inside sorghum mills during warmer months of the calendar year. In addition, *T. castaneum* will reproduce and develop on all of the sorghum fractions included in this study, which are commonly generated during the sorghum milling process. Sanitation and removal of residual materials such as the measured fractions could also help with overall pest management of *T. castaneum*.

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1. Introduction

Sorghum is the fifth most commonly grown grain crop in the world, after wheat, rice, corn, and barley, and is receiving increased utilization for human food consumption (Cardoso et al., 2017). It is drought tolerant, requires low nitrogen fertilizer inputs, and its quality characteristics such as protein content compare favorably with other grain crops, including wheat, corn and rice (Proietti et al., 2015). Historically it was grown in the United States (US) primarily for use as animal feed but there is increasing interest in sorghum as a component as a biofuel and as a component of human food, both for use as gluten-free products and for potential health benefits (Stefoska-Needham et al., 2015; Ferreira et al., 2016). With the increased utilization of sorghum, and potentially increased milling activity in the US, there should also be accompanying concerns regarding stored product insect activity in and around sorghum mills, and if milling fractions and by-products will support insect activity.

Common pests in the wheat milling and rice milling industries include *Tribolium castaneum* (Herbst), the red flour beetle, or the related *T. confusum* duVal, the confused flour beetle (Coleoptera: Tenebrionidae). Both are cosmopolitan pests of stored grains and processed grain products (Campbell et al., 2010a,b; Buckman et al., 2013). Development of these species on raw grains is often limited unless they are associated with a primary feeder, such as *Rhyzopertha dominica* (Fab.), the lesser grain borer, or weevils in the genus *Sitophilus*. *Tribolium castaneum* is much more prevalent in wheat flours, in different processed grain products, or in different edible nut crops that are

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https://doi.org/10.1016/j.jspr.2020.101606
0022-474X/Published by Elsevier Ltd.

stored before distribution to consumers, compared to stored bulk grains. *Tribolium castaneum* can grow and develop on milling fractions and by-products, including those produced in rice milling (Arthur et al., 2019a,b).

For many years the fumigant methyl bromide was used as a control option for this pest, and with the phase-out of methyl bromide continuing use exemptions (CUEs) were granted for use in mills. However, with the withdrawal of methyl bromide in the US, control of flour beetles inside mills has shifted to use of alternative fumigants, heat treatments, residual contact surface treatments, including insect growth regulators (IGRs) and evaluations of the biological pesticide Spinosad, and aerosol insecticides, along with a renewed emphasis on sanitation in conjunction with insecticide applications (Subramanyam et al., 2011; Arthur, 2012; Wijayaratne et al., 2018; Dissanayaka et al., 2020). To date there has been little research regarding *T. castaneum* prevalence in or around sorghum mills, or any research regarding potential growth and development of *T. castaneum* on sorghum milling fractions. The objectives of this study were to determine: 1) if adult females will oviposit in and larvae will develop on selected sorghum milling fractions, 2) if individual larvae can develop to the adult stage on those fractions, and 3) developmental times and successful adult emergence at different holding temperatures. Three separate studies were conducted for this project.

2. Materials and methods

2.1. General information

The *T. castaneum* used in this test were from pesticide-susceptible colonies that had been maintained at the USDA-ARS Center for Grain and Animal Health Research (CGAHR), Manhattan, KS, USA, for more than 30 years. They were reared on a diet of 95% whole wheat flour and 5% Brewer's yeast, and maintained in a Percival incubator (Perry, IA, USA) at 27 ± 1 °C and 69% ± 1 r.h. Six sorghum milling fractions were used in this test: Bran, Coarse Grits, Fine Grits, Flour, Red Dogs, and Shorts. All were obtained from the Department of Grain Science and Industry, Kansas State University, Manhattan, KS. The description of those fractions is listed below, along with a reference for milling of these fractions (Zhao and Ambrose, 2018).

Bran- Particles of sizes greater than 900 μm. It is mostly composed of the outer layers of the grain-pericarp and testa. It primarily contains fibers, protein and minerals. The high content of minerals in the stream contribute to the higher ash content of the bran.

Coarse Grits and Fine Grits- These are particles obtained in the intermediate stages in milling. The particle sizes of the fractions vary from 600 to 900 μm for Coarse Grits and between 193 and 478 μm for Fine Grits. It mostly comprises larger fractions of endosperm and is thereby rich in starch. These are further reduced in the subsequent stages to obtain flour.

Flour- Milling of the grains is aimed at the recovery of the endosperm. The starch-rich endosperm is broken down into finer fraction of size less than 193 μm is flour.

Red Dogs- These are the tailings of the milling consisting of bran, germ and flour. The particle size varies between 193 and 478 μm. The percentage of starch (contributed by endosperm) is lower in this milling fraction compared to other milling fractions. The germ contributes considerable fat content to this fraction.

Shorts- By-products of milling including the bran, germ and flour in the size range of 478–600 μm. The flour (starch) fraction in the shorts is relatively low. Higher protein and ash content of the shorts comes from the bran layer. Fat content of the shorts is mostly due to the germ content.

2.2. Experiment 1

In this test, approximately 50 g of a particular sorghum fraction was placed in a 120-mL vial with a screen lid, along with 20 one-two-week old mixed-sex adult *T. castaneum* from the laboratory colony. There were five replicate treatments for each sorghum milling fraction (36 vials total). After the adults were placed in the vials, all vials were placed in an incubators (Perry, IA, USA) separate from the colony incubator, set at 27 °C-60% r.h. After eight weeks the vials were removed from the incubator and frozen overnight at −18 °C. The next day, the fractions were sieved through either a #16 sieve or a #20 sieve, depending on the specific fraction, to collect adults and larvae (1.18 and 0.84 mm sieve openings, respectively). Each replicate vial contained hundreds of larvae, so for each fraction three groups of 10 larvae were weighed, and an average weight was obtained per group of 10, which was then divided by 10 to obtain an average weight per larva. Then, the total amount of larvae from each vial was weighed and the number of larvae in each replicate for each fraction was then estimated based on the average weight of an individual larvae. The adult count included the original group of 20 parental adults.

2.3. Experiment 2

Results from Experiment 1 showed adults could reproduce and oviposit in the sorghum milling fractions. For this next test, a single neonate 1-2-day old larva was placed in each of twenty-four 60 ml vials containing 1 g of a particular fraction (144 vials total). All vials were placed in a walk-in chamber set at 30 °C and 60% r.h. The vials were examined after 7 weeks, and the presence of a pupa or an adult was recorded.

2.4. Experiment 3

Based on the results of Experiment 2, a third test was initiated. The series of 144 20-ml vials was expanded to include holding temperatures of 22, 27, 32, and 37 °C, in separate incubators (24 replicates for each combination, 576 total vials). Temperature and r.h. was monitored in each incubator using HOBO recording computers (Onset Computers, Bourne, MA, USA). Vials were checked daily beginning two weeks after the vials were placed in the incubator. The date at which an individual neonate reached the adult stage was recorded for each individual vial at each temperature. Data were considered complete for each fraction and temperature by holding all vials for at least two weeks after the last adult was recorded for each temperature-fraction combination.

2.5. Fraction chemical composition

The proximate analysis of the samples was done at Agricultural Experiment Station Chemical Laboratories, University of Missouri-Columbia. Standard American Association of Cereal Chemists (AACC) procedures were followed for the proximate analysis. The reference methods are as follows: ash (AACC 08-01.01), fat (AACC 30-10.01), fiber (AACC 32-10.01), moisture (AACC 44-15.02), protein (AACC 46-30.01), and starch (AACC 76-13.01).

2.6. Statistical analysis

Data for Experiment 1 were analyzed using the Proc Mixed Procedure of the Statistical Analysis System (SAS Version 9.2, Cary, NC, USA). Means were separated using the LSMeans option under the Mixed Procedure. Data for Experiment 2 are reported as raw numbers with no analysis. Data for Experiment 3 were first analyzed using the Mixed Procedure in the SAS to determine the difference between adult emergence with respect to fraction and temperature. The Glimmix Procedure was then used to determine differences between timing of adult emergence among fractions at each temperature. Differences between percentage of total adult emergence at each temperature-fraction combination were analyzed using Fisher's Exact Test in SAS. The range of values was determined as well for each fraction-temperature combination.

Further analysis was done to determine if neonates developed at similar rates with time, by modeling the accumulated percentage of adult emergence as a function of time (days to adult emergence), using methods described by Tavares et al. (2018). Curve fitting was done for four options: Logistic, Compertz, Richards, and Weibull. For most of the temperature-fraction combinations, the Logistic function had the best fit (Supplemental Table 1). Further analysis of similarity of each fraction was conducted by temperature, comparing the curve similarity among each of the fractions. Proc Logistic was used in SAS to determine the logistic fit, with the percent total adults as the response variable, the fraction and emergence day as main effects, and fraction as the Class variation with *param* = ref. Contrasts were performed to directly compare each fraction to one another within temperature, setting the estimate to equal *parm*. The proportion of neonates that did not make it to the adult stage was compared using Proc Corr in SAS to each of the six chemistry components to test the influence of chemistry on neonate development at each temperature.

3. Results

3.1. Experiment 1

Milling fraction was significantly different at $P < 0.01$ for emergence of adults ($F = 59.6$, df = 5,23), larval weight ($F = 7.5$, df = 5,22), and estimated number of larvae ($F = 13.1$, df = 5,22). Bran contained the most adults, but total larval weight and thus estimated larvae were greatest in flour and shorts, respectively (Table 1). The larvae were most likely overcrowded in all the fractions, which limited development to the adult stage and thus the number of adults was lower than expected. Regardless, it was clear that *T. castaneum* could develop on all of the sorghum fractions.

3.2. Experiment 2

Adult emergence was at least 20 out of 24 for all fractions (Table 2). Several fractions contained pupae, which most likely would have advanced to the adult stage if they were given more time to complete development. Neonates survived on 1 g of a fraction, and all fractions supported development from the neonate to the adult stage.

Table 1
Average number of *Tribolium castaneum* F_1 progeny adults, total weight of F_1 larvae in grams (Larval Wt.), and estimated number of F_1 larvae (Est. Larvae) based on weight (means ± SE for all), on six sorghum fractions. Means followed by different lower-case letters denote differences ($P < 0.05$, Tukey Honestly Significant Difference Test, Proc Mixed, SAS).

Fraction	Adults	Larval Wt.	Est. Larvae
Bran	78.4 ± 6.2a	1,487 ± 276a	595.1 ± 110.4b
Coarse Grits	20.4 ± 0.5bc	1,277 ± 180a	392.0 ± 28.9c
Fine Grits	32.6 ± 3.3b	1,506 ± 104a	602.4 ± 41.4b
Flour	19.4 ± 0.2c	1,191 ± 57a	447.6 ± 20.7c
Red Dogs	21.2 ± 0.6bc	797 ± 60b	334.4 ± 35.4c
Shorts	23.8 ± 1.6bc	2,000 ± 121b	833.2 ± 50.5a

Table 2
Number of *Tribolium castaneum* pupae and adults from 24 individual neonate (one-two-day old) larvae in each of 24 replicate vials containing 1 g of six sorghum fractions. Vials held at 27°C-60% r.h. for 7 weeks.

Fraction	Larvae	Pupae	Adults
Bran	1	1	21
Coarse Grits	0	2	22
Fine Grits	0	3	20
Flour	0	3	20
Red Dogs	0	3	21
Shorts	0	2	22

3.3. Experiment 3

Adult emergence was significant at $P < 0.001$ for main effects temperature and fraction $F = 3956.0$, df = 3, 472; $F = 4.9$, df = 5, 472, respectively), but the interaction was not significant ($F = 1.5$, df = 15, 472, $P = 0.064$, all Proc Mixed, SAS). Data for adult emergence in all fractions is shown in Table 3 (Analysis by Proc Glimmix, SAS). At 37 °C, the time to adult emergence ranged from 17.8 to 19.3 days. As temperature decreased to 27 °C, there was a corresponding decrease in time to adult emergence, which ranged from 30.4 to 33.7 days. Emergence time was again greatest in Red Dogs. At 22 °C, time to adult emergence was greatly reduced compared to the other temperatures, with emergence ranging from 80.8 to 90.5 days, and no difference in adult emergence among fractions. At 37 °C, time to adult emergence was greatest on Flour compared to the other fractions. Adult emergence time was greatest at 32 °C on Red Dogs, and emergence ranged between 22.2 and 23.8 days. At 27 °C, time to emergence was also greatest on Red Dogs as well.

The variation in adult emergence is shown by the minimum and maximum development times for emerged adults at all temperatures (Table 4). At 37 and 32 °C, the difference between minimum and maximum emergence times was 3–5 and 3–6 days, respectively. At 27 °C, minimum and maximum emergence times ranged between 6 and 22 days. However, at 22 °C, the difference between the minimum and maximum times was between 33 and 45 days, depending on the specific fraction. This large variation likely contributed to the fact that there was no significant difference in adult emergence among fractions at 22 °C.

Data for percentage of emerged adults was significant for temperature by fraction (P = 0.022), fraction was not significant, ($P = 0.510$), but temperature was significant ($P < 0.001$, all analyses by Fisher's Exact Test). Percentage adult emergence was lowest for Bran and Red Dogs at 22 °C, but there was no difference in emergence among fractions at the other three temperatures (Table 5).

Odds ratio estimates for the effect of days, is 0.62 (lower CL = 0.59, upper CL = 0.66) for 22 °C, 0.32 (0.25, 0.41) for 27 °C, 0.061 (0.028, 0.13) for 27 °C, and 0.11 (0.062, 0.21) for 32 °C. These results indicate that as time increases, the odds of a neonate developing into an adult increased by a factor of 0.62 at 22 °C.

Table 3
Days to adult emergence (Mean ± SE) of *T. castaneum* neonates (1-2-day old larvae) on six different sorghum fractions held at 37, 32, 27, and 22 °C. Means within columns followed by the same letter are significantly different ($P < 0.05$, SAS, Proc. Glimmix, Tukey Adjusted).

Fraction	37 °C	32 °C	27 °C	22 °C
Bran	18.1 ± 0.12bc	22.5 ± 0.30b	30.4 ± 0.40bc	84.9 ± 2.87a
Coarse Grits	19.0 ± 0.26 ab	22.7 ± 0.35a	31.2 ± 0.22bc	85.7 ± 2.88a
Fine Grits	17.8 ± 0.18c	22.2 ± 0.20 ab	32.1 ± 0.47 ab	83.2 ± 2.18a
Flour	19.3 ± 0.32a	22.9 ± 0.23a	31.9 ± 0.70bc	90.5 ± 2.22a
Red Dogs	19.1 ± 0.28 ab	23.8 ± 0.48a	33.7 ± 0.48a	87.7 ± 2.75a
Shorts	18.4 ± 0.21abc	22.7 ± 0.38 ab	30.7 ± 0.60c	80.8 ± 2.24a

Table 4
Minimum and maximum days to adult emergence of *T. castaneum* neonates (1-2-day old larvae) on six different sorghum fractions held at 37, 32, 27, and 22 °C).

Fraction	37 °C		32 °C		27 °C		22 °C	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Bran	17	20	21	26	28	34	67	112
Coarse Grits	17	22	21	25	29	33	68	113
Fine Grits	16	19	21	25	29	38	74	112
Flour	18	23	22	25	28	50	75	108
Red Dogs	17	23	21	27	30	40	69	113
Shorts	17	21	21	26	28	41	68	108

Table 5
Percent adult emergence (out of 24) of *T. castaneum* neonates (1-2-day old larvae) on six different sorghum fractions held at 22, 27, 32, and 37 °C.

Fraction	37 °C	32 °C	27 °C	22 °C
Bran	87.5	100.0	92.7	66.7
Coarse Grits	92.7	87.5	79.2	83.3
Fine Grits	95.8	95.8	92.7	79.2
Flour	79.2	92.7	87.5	70.8
Red Dogs	95.8	92.7	92.7	66.7
Shorts	87.5	92.7	92.7	75.0

However, these odds ratios were calculated on different scales, as the maximum days for adult emergence at 22 °C was 113 days, 40 days for 27 °C, 27 days for 32 °C, and 23 days for 37 °C. When proportion of adults developing over time for all temperatures is extended to 113 days, odds estimates increase for 27 °C to 0.94 (0.93, 0.94), 0.95 for 32 °C (0.95, upper CL = 0.96), and 0.96 (0.96, 0.97) for 37 °C.

Logistic comparison between fraction reveals considerable variation when comparing fractions within each temperature (Table 6). For example, adult development compared between Flour and Fine Grits is significantly different for all temperatures (Fig. 1). Similar patterns are found for all temperatures for Bran compared to Flour and Fine Grits compared to Red Dogs. In comparison, Coarse Grits and Red Dogs only showed significantly different patterns of adult emergence at 22 °C and not any other temperatures (Table 6).

The main effect fraction was significant for the six measured chemical components (Table 7). Ash, fat, and fiber content were greatest in Bran compared to the fractions, while starch was 4-5-fold lower in Bran. The Pearson correlation analysis showed some effects of chemical composition on neonate development. Fat, ash, and fiber had a negative correlation to proportion of adults that did not emerge at 32 °C (Table 8). Moisture was also significant for the proportion of neonates that did not emerge to adults at 22 °C (Table 8).

4. Discussion

In the initial phases of this experiment, it was clear that parental *T. castaneum* could survive and reproduce on the milled sorghum fractions that were tested. All sorghum fractions supported development of individual neonate *T. castaneum* to some extent on all temperatures ranging from 22 to 37 °C. In an earlier study with neonate *T. castaneum* exposed on rice milling fractions, in which neonates survived to the adult stage, the time to emergence was greatly extended at 22 °C compared to 27, 32, and 37 °C (Arthur et al., 2019a). In the current study with sorghum the time to adult emergence of neonates exposed at 22 °C was extended to almost four months, with considerable variation in emergence time among individual neonates. In contrast to the earlier study with milled rice fractions, the percentage of adult emergence was reduced among the sorghum fractions at 22 °C compared to the earlier study with the rice fractions.

Tribolium castaneum is considered to be a major pest of wheat mills and rice mills, and there have been numerous recent studies documenting the presence of this insect in and around milling and storage facilities in the midwestern and southern USA (Arthur et al., 2015; McKay et al., 2017, 2019). Populations inside facilities are considered to be more or less permanently established or perhaps arising from infested material being brought into a site, rather than arising from immigration from outside populations (Buckman et al., 2013). Populations inside facilities can even persist through the winter months, again indicating the presence of resident infestations (Campbell, 2013; Semeao et al., 2013a,b; Arthur et al., 2014). Thus, it is evident that *T. castaneum* could also be prevalent in sorghum mills, and there should be an emphasis on monitoring and control, especially with the potential expansion of sorghum and value-added sorghum products.

There is evidence from previous studies that *T. castaneum* is susceptible to cold temperatures, with minimal development at temperatures which support development of other stored product beetles (Wijayarante and Fields, 2010). Howe (1965) published an early comprehensive review of the minimum and optimal developmental temperatures for stored product insects. *Tribolium castaneum* was classified as a “Species Needing High Temperature”, with minimum and optimum developmental temperatures of 22 °C

Table 6
Logistic comparison between fractions at each temperature. DF = 1 in all cases.

Comparison	22 °C		27 °C		32 °C		37 °C	
	Wald	P	Wald	P	Wald	P	Wald	P
Bran vs Coarse Grits	56.8	<0.001	24.0	<0.001	14.4	<0.001	0.5	0.49
Bran vs Fine Grits	78.2	<0.001	10.2	0.001	0.02	0.88	8.7	0.003
Bran vs Flour	12.6	<0.001	23.4	<0.001	9.3	0.003	12.0	<0.001
Bran vs Red Dogs	4.8	0.03	24.6	<0.001	20.8	<0.001	0.04	0.85
Bran vs Shorts	72.4	<0.001	4.8	0.03	11.3	<0.001	0.4	0.54
Coarse Grits vs Fine Grits	3.4	0.06	5.2	0.02	15.0	<0.001	11.8	<0.001
Coarse Grits vs Flour	98.1	<0.001	0.01	0.92	1.3	0.26	9.0	0.003
Coarse Grits vs Red Dogs	82.0	<0.001	0.01	0.92	2.0	0.15	0.8	0.38
Coarse Grits vs Shorts	1.8	0.18	10.8	<0.001	0.4	0.52	0.01	0.94
Fine Grits vs Flour	119.0	<0.001	4.8	0.03	9.7	0.002	25.5	<0.001
Fine Grits vs Red Dogs	103.5	<0.001	5.6	0.02	21.4	<0.001	7.8	0.005
Fine Grits vs Shorts	0.3	0.62	1.3	0.25	12.0	<0.001	11.5	<0.001
Flour vs Red Dogs	2.0	0.16	0.04	0.85	5.9	0.02	12.9	<0.001
Flour vs Shorts	113.5	<0.001	10.2	0.001	0.3	0.62	9.3	0.002
Red Dogs vs Shorts	97.7	<0.001	11.3	<0.001	4.0	0.04	0.6	0.43

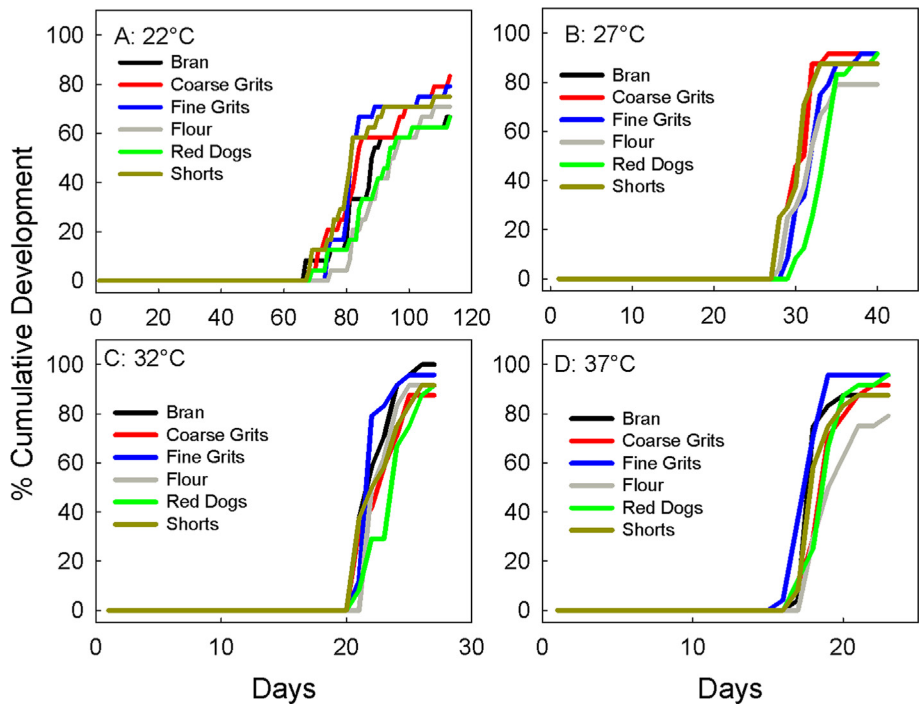

Fig. 1. Percentage cumulative adult development (y-axis) with respect to days to adult emergence (x-axis) of neonates exposed on six sorghum fractions at 22 °C (A), 27 °C (B), 32 °C (C), at 37 °C (D). Note x-axis scale is different for each temperature, legend is the same for all sub-figures.

and 32–35 °C, respectively. Fields (1992) describes *T. castaneum* as a cold-susceptible species. In a study whereby parental *T. castaneum* adults were exposed on brown rice and rice flour, there was little population growth even after one year when the rice and flour were held at 22 °C (Arthur et al., 2019b). Thus, it appears that low temperature could be integrated with other management strategies for *T. castaneum* in sorghum mills and value-added production facilities.

Consideration of timing of adult emergence is also important for monitoring and managing insect infestations. As discussed above, across temperatures, the timing of emergence was significantly longer at cooler temperatures. Within each temperature, curves of cumulative adult emergence over time showed significant variation across fractions as well. The difference in timing is important to dissect since average days to emergence was not significantly different among fractions at 22 °C, but for example, adult emergence on Flour did not start at the same time as Bran, Coarse Grits, Fine Grits, and Shorts, but then had a steeper curve of cumulative adult emergence. Similarly, we can compare specific fractions such as Bran and Coarse grits. These two fractions differed in timing of adult emergence at 22, 27, and 32 °C but were not significantly different in average days to emergence. In contrast, Coarse Grits and Shorts do not significantly differ at 22, 32, and 37 °C in timing of adult emergence and do not differ in average emergence time at these temperatures. Therefore, understanding how emergence curves differ can elucidate broader patterns of adult emergence on these different fractions and can help guide managers in monitoring fractions more accurately.

Table 8
Pearson correlations of fraction chemistry compared to proportion of neonates that did develop to the adult stage at each temperature.

Component	Test Value	22 °C	27 °C	32 °C	37 °C
Ash	Pearson	0.55	−0.47	−0.77	0.05
	P	0.26	0.35	0.07	0.92
Fat	Pearson	0.62	−0.55	−0.80	0.04
	P	0.19	0.26	0.06	0.93
Fiber	Pearson	0.53	−0.54	−0.73	0.03
	P	0.28	0.27	0.10	0.96
Moisture	Pearson	−0.85	0.44	0.31	−0.16
	P	0.03	0.38	0.54	0.76
Protein	Pearson	0.60	−0.57	−0.26	−0.23
	P	0.21	0.24	0.63	0.67
Starch	Pearson	−0.64	0.47	0.73	−0.14
	P	0.17	0.35	0.10	0.79

Table 7
Percentage composition (Mean ± SE, n = 2) of chemical constituents in sorghum fractions. Means within columns followed by different lower-case letters are significantly different ($P < 0.05$, Tukey Honestly Significant Difference Test, Proc Mixed, SAS).[1]

Fraction	Ash	Fat	Fiber	Moisture	Protein	Starch
Bran	7.0 ± .04a	14.0 ± 0.13a	9.4 ± 0.02a	11.1 ± 0.12c	14.8 ± 0.13b	16.1 ± 0.07c
Coarse Grits	1.3 ± .17c	0.8 ± 0.28e	1.6 ± 0.03d	14.0 ± 0.08a	10.4 ± 0.04d	84.8 ± 0.86a
Fine Grits	1.2 ± .02c	1.6 ± 0.03d	1.5 ± 0.04d	14.1 ± 0.25a	8.8 ± 0.20e	88.5 ± 0.38a
Flour	0.9 ± .01d	0.8 ± 0.04e	0.6 ± 0.01e	12.6 ± 0.10b	8.6 ± 0.05e	82.9 ± 2.42a
Red Dogs	2.0 ± .01b	4.0 ± 0.04a	2.9 ± 0.02c	10.9 ± 0.06d	15.2 ± 0.06a	69.4 ± 1.28b
Shorts	1.3 ± .00c	3.0 ± 0.11c	3.8 ± 0.07b	11.3 ± 0.03c	14.1 ± 0.12c	70.8 ± 0.44b

[1] Fraction was significant at $P < 0.001$ for each constituent, df is always 5,5, F values in order from Ash to Starch are 1,208.7, 1177.0, 8447.7, 356.7, 710.6, and 545.2 (Proc Mixed, SAS).

The chemical composition of the fractions did have some effect on development of *T. castaneum* neonates, especially at 22 °C, though not as much as expected given the variation in composition among the fractions. In this test, an individual neonate was exposed on 1 g of a fraction, which apparently provided enough nutrition for the neonates to complete development on the fractions regardless of chemical composition. The lowest temperature could have imposed a stress on development that was exacerbated by the lower moisture content on some of the fractions. The negative correlations to fat, fiber and ash at 32 °C may suggest the effect of anti-nutrients on development as these grain components are primarily found in the outer layers of the grain or the germ. However, the fact that these factors were not correlated to the proportion of neonates at all temperatures complicates any conclusions that can be drawn between the composition of the milling fractions and neonate development. Further research may be needed to elucidate the effects of milling fraction chemistry and composition on development of *T. castaneum*.

Previous trials have also shown that *T. castaneum* can develop to the adult stage on milled brown rice, which has lost the protective husk, with progeny production dependent on temperature (Arthur et al., 2019b). The primary feeder *R. dominica* can develop on different sorghum varieties, though the level of progeny production may depend on inherent qualities such as kernel hardness, protein content, and starch content (Arthur et al., 2020). Sorghum milling facilities could have storage bins containing raw sorghum grains, similar to wheat and rice mills, thus it would be beneficial to determine susceptibility of raw sorghum to *T. castaneum* either alone or in combination with *R. dominica*. Evidence from studies with Pakistani sorghum varieties show that *T. castaneum* can survive on raw sorghum and also cause feeding damage and quality deterioration (Majeed et al., 2016). Thus, assessments of the ability of *T. castaneum* to survive on common US sorghum varieties would be of benefit to the expanding sorghum industry in this country.

Declaration of competing interest

The authors declare no conflict of interest with data associated with this publication.

CRediT authorship contribution statement

F.H. Arthur: Conceptualization, Data curation, Writing - original draft. **S.R. Bean:** Data curation, Writing - original draft. **D. Smolensky:** Data curation, Writing - original draft. **A.R. Gerken:** Data curation, Formal analysis, Writing - original draft. **K. Siliveru:** Writing - original draft. **E.D. Scully:** Writing - original draft. **N. Baker:** Data curation.

Acknowledgments

We would like to thank Jaymi Petersen for excellent technical assistance. This paper reports the results of research only. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA or by Kansas State University. The USDA and Kansas State University are equal opportunity employers and providers. This paper is assigned contribution # 20-201-J of the Kansas Agricultural Experiment Station.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jspr.2020.101606.

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