Diapause Response of the Boll Weevil (Coleoptera: Curculionidae) to Selected Diets[1]

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Abstract The boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), once the dominant pest of cotton (*Gossypium* spp.) in the United States, is now the most serious pest of South American cotton. Despite eradication efforts in the United States, intractable populations in southern Texas and northern Mexico remain a threat to adjoining regions. A key to eliminating this pest from the subtropics may lie in better understanding mechanisms facilitating survival through the non–cotton season. We examined the diapause response to diet regimes by evaluating the influences of food type (square, boll), size, and replacement interval, under photoperiod (13:11 [L:D] h) and temperature conditions (29.4°C) considered to suppress diapause. Female weevils exhibited diapause characters earlier than did males on all diets, and physiological status was not reliably evaluated until adults were 9 d old. When squares fed to groups of weevils were replaced thrice weekly, most weevils responded with symptoms of starvation instead of diapause. In other feeding regimes, incidence of diapause increased with increasing food development, which may reflect the weevil perception of host maturity. These results are consistent with accounts of seasonality of diapause and host utilization in the subtropics and tropics, as well as accounts of late-season weevil ecology in temperate regions prior to the widespread adoption of mechanized harvest and determinant cotton cultivars. These findings, combined with earlier demonstrations of extended host-free longevity and lack of a photoperiod response, identify food characteristics as important determinants of boll weevil reproductive diapause irrespective of other environmental cues.

Key Words *Anthonomus grandis grandis*, diapause, *Gossypium*, cotton, diet

The boll weevil, *Anthonomus grandis grandis* Boheman, was once the most important pest of cotton (*Gossypium* spp.) in the United States. Although eradication programs have eliminated the weevil as an economic pest over most of the Cotton Belt, the Lower Rio Grande Valley of Texas and Mexico remains infested, and that region is the putative source of periodic reinfestations of bordering production regions. The boll weevil is also the most serious pest of South American cotton (de Oliveira et al. 2016, Scataglini et al. 2006), where improved management is under intense investigation.

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One factor hampering efforts to manage the boll weevil in the tropics and subtropics is the controversy regarding the mechanisms and dynamics of boll weevil overwintering survival. Since the report of Brazzel and Newsom (1959), most researchers have accepted the primary mechanism of overwintering is adult reproductive diapause. Earle and Newsom (1964) identified photoperiod and temperature as important determinants of diapause in the weevil. Although the report by Earle and Newsom (1964) motivated numerous later studies, most studies examining photoperiodic or thermal induction of boll weevil diapause have yielded inconsistent and sometimes conflicting results. Spurgeon and Raulston (1998, 2006) suggested many of these inconsistencies were attributed to variations in the diets employed, which were typically poorly described or controlled, and failure to account for differences in physiological age of the weevils where experimental treatments involving different temperatures were assessed at a fixed chronological age, or at widely variable ages. Another source of variation is the criteria used to distinguish diapause, which have varied among studies (Spurgeon et al. 2003). In addition, most of the work on boll weevil diapause has not featured true replication of the treatments, or has used experimental designs that did not permit examination of interactions among putative diapause-inducing cues (Spurgeon and Suh 2017a). More recently, Spurgeon and Raulston (2006) could not demonstrate an influence of photoperiod or temperature on the incidence of diapause in the weevil. Instead, they found that feeding regime was the most influential factor, although temperature affected the timing with which reproductive and diapause characters appeared. Spurgeon and Suh (2017a) reported that, under a putative diapause-suppressing photoperiod, temperature influenced the timing with which the diapause characters appeared but not the incidence of diapause.

Numerous reports have implied a role of adult diet in the induction of diapause in the boll weevil, either alone (Graham et al. 1979, Keeley et al. 1977, Lloyd and Merkl 1961) or in combination with environmental cues (Bradley and Phillips 1978, Cobb and Bass 1968, Rummel and Summy 1997). In addition, a high incidence of diapause (Spurgeon and Raulston 2006, Spurgeon and Suh 2017a), presence of a diapause-associated storage protein (Lewis et al. 2002), extended host-free survival (Spurgeon et al. 2008, Spurgeon and Suh 2017a, Suh and Spurgeon 2006), and high levels of overwinter survival (Westbrook et al. 2003) have been obtained by manipulating the adult diet. Except for Spurgeon et al. (2008), these studies utilized photoperiods reputed to suppress diapause. No report has explicitly examined diapause induction in relation to size and developmental maturity of the food items (cotton squares and bolls). We examined the influences of diet (food type, size, and maturity) on the incidence of diapause in the boll weevil.

Materials and Methods

Responses to adult diet were examined in three experiments. In each experiment, weevils were reared to adulthood from flower buds (squares) collected from cotton plants in Central Texas before they had abscised. Infested squares were held within environmental chambers at 29 ± 2°C with a photoperiod of 13:11 (L:D) h. A putative diapause-suppressing photoperiod was used because the day length while cotton is maturing in subtropical Texas, when diapause would be naturally induced, is >12 h. Squares were inspected for pupae beginning 5–6 d after collection, and pupae were harvested twice daily after they were initially observed. Harvested pupae were held on moistened vermiculite within 100 × 15-mm Petri plates until adult eclosion. The petri plates were held under the same conditions as the infested squares. Twice daily, the petri plates were examined for newly eclosed adults, which were sexed by the tergal notch method (Sappington and Spurgeon 2000) and assigned to experimental treatments.

Squares and bolls were obtained from the field when they were available; otherwise, they were obtained from greenhouse-grown plants. Squares and bolls were collected into sealable plastic bags containing reverse-osmosis water to prevent desiccation, and were rinsed and drained in the laboratory. Rinsed food items were stored in a laboratory refrigerator (≤3 d) before use. Only squares and bolls free of external injuries or blemishes were fed to the weevils.

Diapause status was determined by dissection as described by Spurgeon et al. (2003). Hypertrophied fat bodies were predominantly bright white, in distinct globules, and obscured most or all of the alimentary and reproductive tracts. Ovaries of females were examined for reproductive development (presence of vitellogenic oocytes or eggs) and for follicular relics indicative of previous oviposition (Grodowitz and Brewer 1987) or oosorption (Spurgeon et al. 2003). Testes were considered atrophied if they were small and opaque from fat. Only weevils exhibiting fat body hypertrophy and undeveloped (females) or atrophied (males) gonads were classed as diapausing. Females were also examined for numbers of combined vitellogenic oocytes (containing yolk) and eggs, and presence of oosorption.

In each experiment, mixed-sex groups of 25 weevils (12 ♂♂, 13 ♀♀) were held in 473-cm^3 cardboard cartons closed with screened lids. A carton of weevils was the experimental unit. In addition to the assigned diet, each carton was provisioned with water in a plastic cup (29.5 ml) closed by a lid that was penetrated by a cotton wick. The cartons were held in environmental chambers maintained at 29.4 ± 1°C with a 13:11 (L:D) h photoperiod. Square diets were fed at a rate of one square per five weevils (five squares per carton). When >2 weevils within a carton died before dissection, the number of squares was reduced to maintain approximately the assigned feeding rate. This square diet is known to induce diapause in the weevil (Spurgeon and Raulston 2006) and is typical of diets used in earlier studies (Harris et al. 1969, Lloyd et al. 1967, McCoy et al. 1968, Tingle and Lloyd 1969). Boll diets were fed at a rate of two bolls per 25 weevils. Bracteoles were removed from food items. Repetitions of each experiment represented separate field collections of weevils.

Influence of square size. Diet treatments included small (5–7-mm diameter) and large squares (>7–9-mm diameter). Small squares represented development up to about 1/3-grown, whereas large squares were between 1/3-grown and candle stage when the petals elongate. Food squares were replaced daily. Diapause was assessed by dissection at 6, 9, and 12 d of adult age. Each treatment combination (square size × age at dissection) was represented by a separate carton of weevils. Treatment combinations were replicated three times in each of two repetitions of the experiment, representing totals of 68–78 weevils of each sex per treatment combination.

Comparisons of square and boll diets. Diet treatments included large squares (6–9-mm diameter) replaced daily, large squares replaced thrice weekly, and medium-sized bolls (17–22-mm diameter) replaced thrice weekly. Diapause was assessed at 6, 9, and 12 d of adult age. Treatment combinations were replicated three times (cartons) in each of two experimental repetitions. Total numbers of weevils representing each sex and treatment combination ranged from 61 to 77.

Influence of boll size. Spurgeon and Suh (2009) observed a low incidence of diapause and relatively high pheromone production among single male weevils provided bolls 12–23-mm diameter. In contrast, Westbrook et al. (2003) observed high levels of diapause and overwinter survival for weevils fed bolls 17–25-mm diameter, and Lewis et al. (2002) and Spurgeon and Suh (2017a) obtained high levels of diapause by feeding weevils bolls of 20–25-mm diameter. Therefore, we examined the diapause response to three boll size classes (10–15-, 15–20-, and 20–25-mm diameter). Bolls were replaced every other day, and diapause was assessed at 6, 9, and 12 d of adult age. Each of four experimental repetitions included a single carton of weevils for each treatment combination (boll size × age at dissection), representing totals of 43–51 weevils of each sex for each treatment combination.

Statistical analyses. All analyses were conducted using the GLIMMIX procedure of SAS (SAS Institute 2012). In each experiment the incidence of diapause, oosorption, and vitellogenesis (including eggs) were examined using mixed models with a binomial distribution. Total numbers of vitellogenic oocytes plus eggs were similarly examined, but using a Poisson distribution. Because females induced into diapause do not exhibit ovary development, and oosorption can be observed only in vitellogenic oocytes or eggs, analyses of oosorption and of numbers of oocytes and eggs included only females exhibiting vitellogenesis. Analyses of the occurrence of diapause, vitellogenesis, oosorption in the third (boll size) experiment, and oocyte numbers in the first (square size) experiment used conditional models estimated by the Laplace method. In remaining analyses, conditional models failed to provide stable covariance estimates or did not converge, so marginal models were used. Marginal models used a bias correction (the DDFM=KR option of the MODEL statement) to adjust denominator degrees of freedom.

Analyses of diapause incidence included fixed effects of diet regime, weevil sex, age at dissection, and their interactions. The first two experiments (square size, squares versus bolls) used a generalized randomized complete block design with repetition of the experiment as a random (blocking) effect. The random term of repetition × diet regime × age served as the error term for tests of diet, age, and their interaction. The random term of repetition × diet regime × age × sex was the error term for the fixed effect of sex and for interactions containing sex. The third experiment (boll size) used a randomized complete block design and events/trials syntax. Fixed effects were the same as for the other experiments. Experimental repetition was a random effect, and the random interaction of repetition × diet × age was the error term for tests of diet regime, age, and their interaction. Residual was the error term for sex and interactions containing sex. Where only female characters were examined (yolk presence, oosorption, oocyte numbers), the models excluded the fixed effect of sex and its interactions.

When model convergence was prevented by complete separation (e.g., zero incidence of diapause for one sex at one age), variances were stabilized and convergence was achieved by rescaling each zero count to 0.01. When interactions appeared nonnegligible they were explored by examining their simple effects (SLICE= and SLICEDIFF= options of the LSMEANS statement). In those cases, main effect tests are not interpreted because they are uninformative (Stroup 2013). For pairwise comparisons of more than two means, *P*-values were adjusted for multiplicity (SIMULATE option of the LSMEANS statement), and means and estimated SE were obtained on the data scale using the ILINK option.

Results

Influence of square size. Interactions between weevil sex and diet, and sex and age, indicated the respective effects of diet and age on the incidence of diapause were conditional on weevil sex (Table 1). Simple effects of the diet × sex interaction indicated a greater diapause response by both weevil sexes to large squares compared with small squares (Table 1; Fig. 1A, B). Comparisons within diets indicated a higher incidence of diapause for males than for females on large squares, whereas the opposite was observed for small squares (Table 1; Fig. 1A, B). Simple effects of the age × sex interaction indicated a higher probability of diapause for females compared with males at 6 d of adult age, but no difference between sexes at 9 or 12 d (Table 1; Fig. 1A, B). Comparisons among ages within sexes indicated no differences in diapause incidence for females (Table 1; Fig. 1A), whereas the incidence of diapause for males at 6 d was lower compared with 9 (adjusted-$P = 0.01$) or 12 d (adjusted-$P < 0.01$, Fig. 1B).

The probability of vitellogenesis varied with female age (diet × age interaction, Table 1). Simple effect tests did not indicate an age effect within diets (Table 1), or differences between diets at ages <12 d (Table 1; Fig. 1C). However, the probability of oocytes with yolk was higher for females fed small squares compared with large squares at 12 d (Table 1; Fig. 1C).

Oosorption was more prevalent for females fed large squares compared with small squares only at 6 d of age (Table 1; Fig. 1D). An age effect was observed for both diets (Table 1). For females fed small squares, oosorption was more prevalent at 9 d compared with 6 or 12 d ($0.02 \leq$ adjusted-$P \leq 0.04$; Fig. 1D). For females fed large squares the incidence of oosorption was lower by 12 d compared with 6 (adjusted-$P = 0.02$) or 9 d of age (adjusted-$P < 0.01$; Fig. 1D), whereas the incidence of oosorption was similar at 6 and 9 d (adjusted-$P = 0.77$).

The influence of diet on combined numbers of oocytes and eggs exhibited by reproductive females ($n = 33$ to 59 per diet × age combination) varied with age (diet × age; $F = 3.75$; df = 2, 28; $P = 0.04$). Simple effect tests indicated an influence of age for both small ($F = 4.31$; df = 2, 28; $P = 0.02$) and large squares ($F = 9.53$; df = 2, 28; $P < 0.01$). The mean (± SE) number of oocytes per female fed small squares was higher at 6 d (10.3 ± 0.81) compared with 9 d (7.8 ± 0.60), and the mean at 12 d (9.0 ± 0.64) was not different from the other ages ($0.25 \leq$ adjusted-$P < 0.33$). In contrast, the number of oocytes for females fed large squares was higher at 12 d (13.3 ± 1.1) compared with 9 d (8.6 ± 0.70; adjusted-$P < 0.01$) and the mean at 6 d (10.9 ± 0.78) was intermediate ($0.05 <$ adjusted-$P \leq 0.09$). Differences between

Table 1. Analyses of incidence of diapause, vitellogenesis, and oosorption for boll weevil adults fed small (S; 5–7-mm diameter) or large (L; >7–9-mm diameter) squares for 6, 9, or 12 d at 29.4°C with a 13:11 (L:D) h photoperiod.

	Diapause				Vitellogenesis			Oosorption		
Effect	*F*	df	*P*	Effect*	*F*	df	*P*	*F*	df	*P*
Diet	34.9	1, 29	<0.01	Diet	2.34	1, 29	0.14	9.72	1, 22.3	<0.01
Age	5.98	2, 29	<0.01	Age	0.02	2, 29	0.98	9.74	2, 22.1	<0.01
Diet × age	0.21	2, 29	0.81	Diet × age	3.45	2, 29	0.045	3.35	2, 21.6	0.054
Sex	1.68	1, 30	0.20	Diet (6 d)**	1.11	1, 29	0.30	13.6	1, 27.9	<0.01
Diet × sex	14.4	1, 30	<0.01	Diet (9 d)**	1.09	1, 29	0.31	2.74	1, 13.9	0.12
Age × sex	3.44	2, 30	0.045	Diet (12 d)**	7.09	1, 29	0.01	0.00	1, 26.0	0.95
Diet × age × sex	0.15	2, 30	0.86	Age (L)**	1.90	2, 29	0.17	6.40	2, 18.7	<0.01
Diet (♀)**	9.67	1, 30	<0.01	Age (S)**	1.59	2, 29	0.22	5.58	2, 21.9	0.01
Diet (♂)**	35.5	1, 30	<0.01	—	—	—	—	—	—	—
Sex (S)**	8.42	1, 30	<0.01	—	—	—	—	—	—	—
Sex (L)**	6.89	1, 30	0.01	—	—	—	—	—	—	—
Sex (6 d)**	5.55	1, 30	0.03	—	—	—	—	—	—	—
Sex (9 d)**	0.08	1, 30	0.78	—	—	—	—	—	—	—
Sex (12 d)**	1.28	1, 30	0.27	—	—	—	—	—	—	—
Age (♀)**	1.09	2, 30	0.35	—	—	—	—	—	—	—
Age (♂)**	6.32	2, 30	<0.01	—	—	—	—	—	—	—

* Analyses of yolk and oosorption incidence use the same model effects.

** Simple effect tests of a main effect within a level of an interacting effect (in parentheses).

diets occurred only at 12 d ($P < 0.01$), when females fed large squares contained more oocytes than females fed small squares.

Comparisons of square and boll diets. The influence of feeding regime on the incidence of diapause was conditional on weevil age and sex (diet × age × sex interaction; Table 2). Simple effect tests indicated a significant effect of feeding regime within each combination of weevil age and sex (Table 2; Fig. 2A, B). The probability of diapause for females fed squares daily was similar to that of females fed bolls at all ages ($0.33 \leq$ adjusted-$P \leq 0.94$; Fig. 2A). However, diapause occurred more frequently on both of these diets at all ages compared with females fed squares thrice weekly (adjusted-$P \leq 0.03$). At 6 d of age the diapause response of males fed bolls was greater compared with either square diet (adjusted-$P \leq 0.01$; Fig. 2B). At ages of 9 or 12 d, the probability of diapause for males fed bolls was similar to that of males fed squares daily ($0.32 \leq$ adjusted-$P \leq 0.91$), but both diets

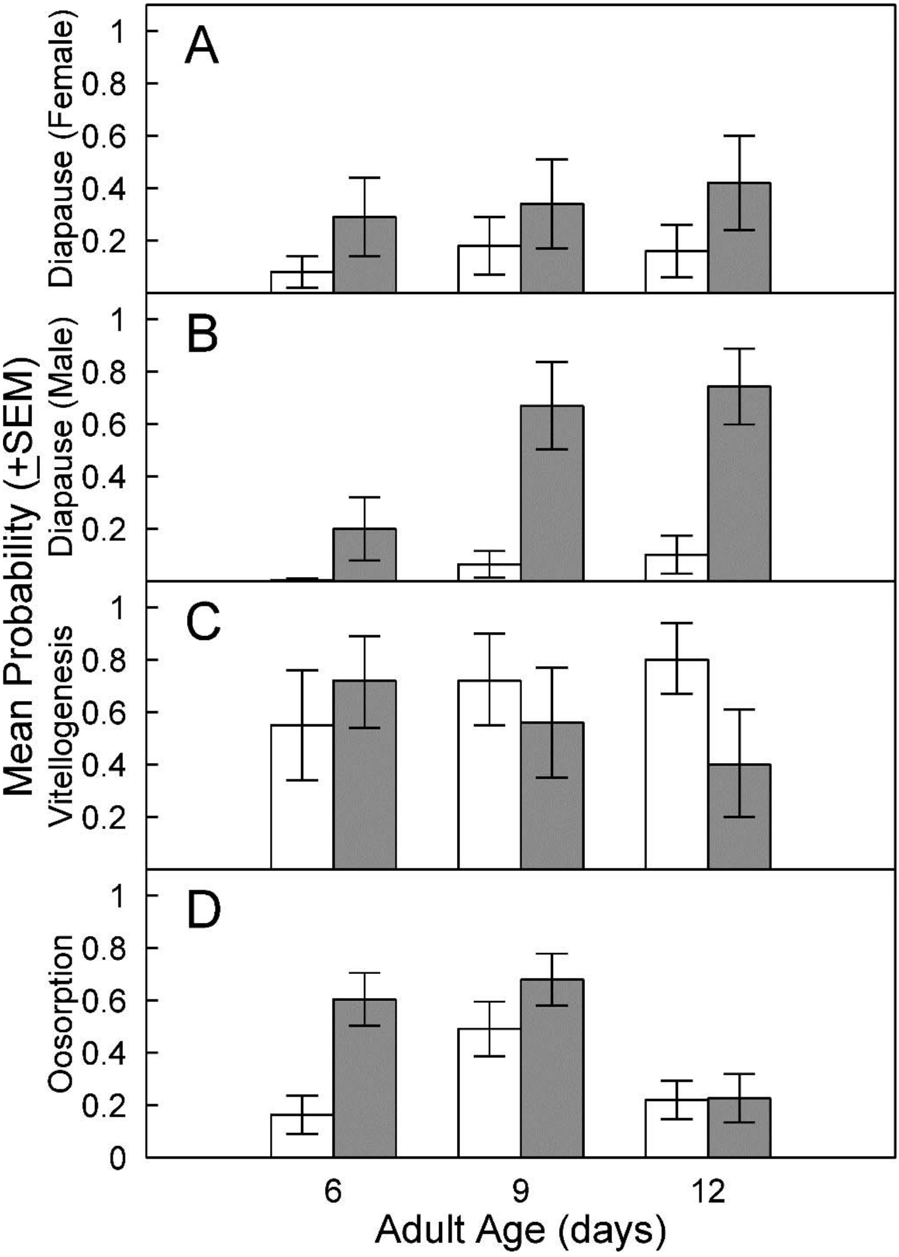

Fig. 1. **Mean probability (± SE) of female diapause (A), male diapause (B), vitellogenesis (C), and oosorption (D) for boll weevils dissected at different ages after feeding as groups on small cotton squares (white bars) or large squares (shaded bars) at 29.4°C and a 13:11 (L:D) h photoperiod.**

Table 2. Analyses of incidence of diapause, vitellogenesis, and oosorption for boll weevil adults fed squares (6–9-mm diameter) daily (SD), thrice weekly (STW), or bolls (17–22-mm diameter) thrice weekly (BTW) for 6, 9, or 12 d at 29.4°C with a 13:11 (L:D) h photoperiod.

	Diapause				Vitellogenesis			Oosorption		
Effect	***F***	**df**	***P***	**Effect***	***F***	**df**	***P***	***F***	**df**	***P***
Diet	55.5	2, 44	<0.01	Diet	1.08	2, 44	0.35	1.24	2, 31.9	0.30
Age	7.68	2, 44	<0.01	Age	7.38	2, 44	<0.01	2.41	2, 31.8	0.11
Diet × age	2.54	4, 44	0.053	Diet × age	2.82	4, 44	0.04	1.05	4, 31.6	0.40
Sex	62.1	1, 45	<0.01	Diet (6 d)**	0.56	2, 44	0.58	—	—	—
Diet × sex	1.64	2, 45	0.20	Diet (9 d)**	5.46	2, 44	<0.01	—	—	—
Age × sex	11.2	2, 45	<0.01	Diet (12 d)**	2.20	2, 44	0.12	—	—	—
Diet × age × sex	1.92	4, 45	0.12	Age (BTW)**	1.14	2, 44	0.33	—	—	—
Diet (♀ 6 d)**	21.7	2, 45	<0.01	Age (SD)**	3.60	2, 44	0.04	—	—	—
Diet (♀ 9 d)**	18.2	2, 45	<0.01	Age (STW)**	8.14	2, 44	<0.01	—	—	—
Diet (♀ 12 d)**	7.78	2, 45	<0.01	—	—	—	—	—	—	—
Diet (♂ 6 d)**	8.95	2, 45	<0.01	—	—	—	—	—	—	—
Diet (♂ 9 d)**	17.5	2, 45	<0.01	—	—	—	—	—	—	—
Diet (♂ 12 d)**	16.2	2, 45	<0.01	—	—	—	—	—	—	—
Sex (BTW 6 d)**	25.3	1, 45	<0.01	—	—	—	—	—	—	—
Sex (BTW 9 d)**	1.97	1, 45	0.17	—	—	—	—	—	—	—
Sex (BTW 12 d)**	4.27	1, 45	0.045	—	—	—	—	—	—	—
Sex (SD 6 d)**	44.3	1, 45	<0.01	—	—	—	—	—	—	—
Sex (SD 9 d)**	1.68	1, 45	0.20	—	—	—	—	—	—	—
Sex (SD 12 d)**	1.08	1, 45	0.30	—	—	—	—	—	—	—
Sex (STW 6 d)**	8.43	1, 45	<0.01	—	—	—	—	—	—	—
Sex (STW 9 d)**	8.09	1, 45	<0.01	—	—	—	—	—	—	—
Sex (STW 12 d)**	8.26	1, 45	<0.01	—	—	—	—	—	—	—
Age (♀ BTW)**	0.88	2, 45	0.42	—	—	—	—	—	—	—
Age (♂ BTW)**	3.77	2, 45	0.03	—	—	—	—	—	—	—
Age (♀ SD)**	1.32	2, 45	0.28	—	—	—	—	—	—	—
Age (♂ SD)**	19.9	2, 45	<0.01	—	—	—	—	—	—	—
Age (♀ STW)**	2.20	2, 45	0.12	—	—	—	—	—	—	—
Age (♂ STW)**	3.48	2, 45	0.04	—	—	—	—	—	—	—

* Analyses of yolk and oosorption incidence use the same model effects.

****** Simple effect tests of a main effect within a level of an interacting effect (in parentheses).

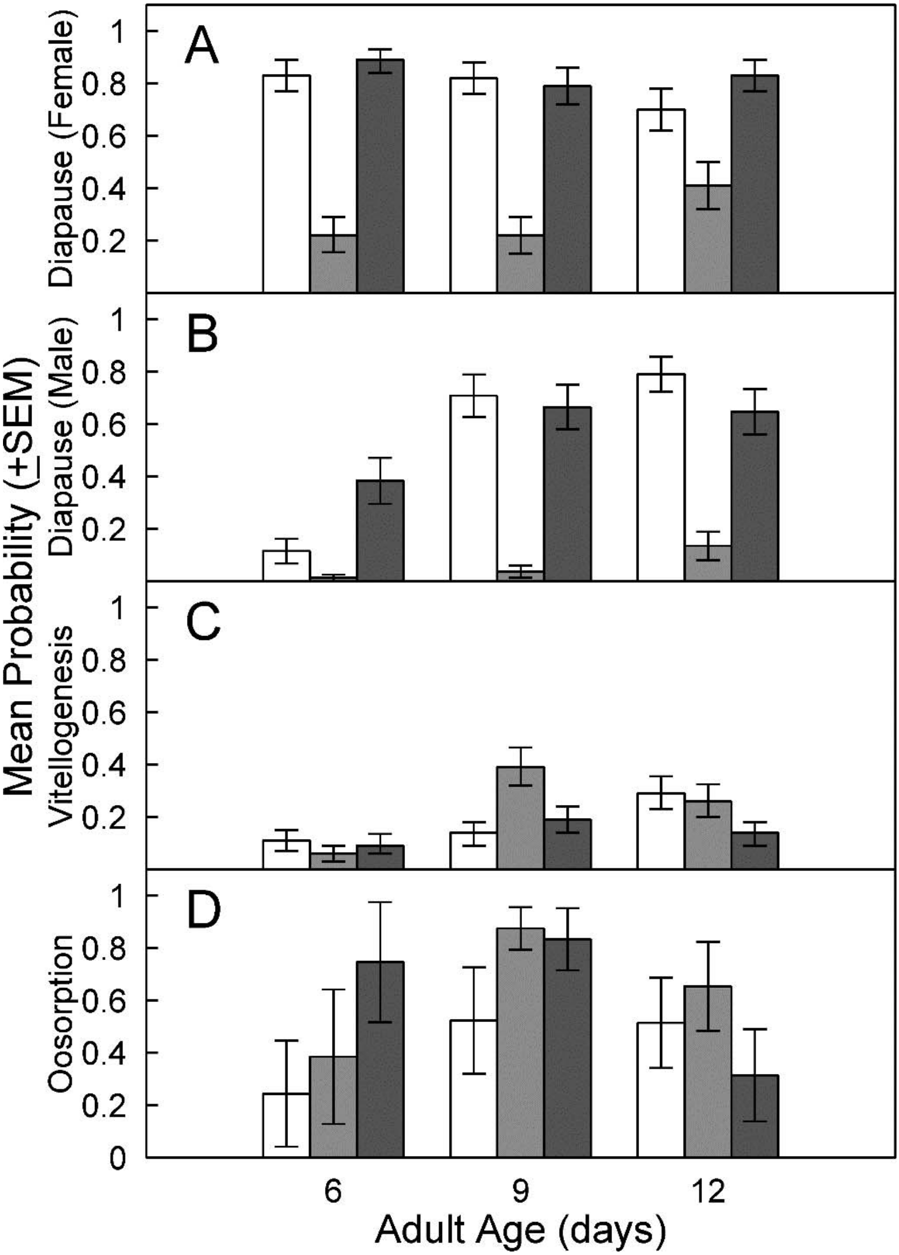

Fig. 2. **Mean probability (± SE) of female diapause (A), male diapause (B), vitellogenesis (C), and oosorption (D) for boll weevils dissected at different ages after feeding as groups on cotton squares replaced daily (white bars), squares replaced thrice weekly (medium gray bars), or bolls replaced thrice weekly (dark gray bars) at 29.4°C and a 13:11 (L:D) h photoperiod.**

produced a higher incidence of diapause compared with males fed squares thrice weekly (adjusted-$P < 0.01$; Fig. 2B).

At 6 d of age the probability of diapause was higher for females than for males on all diets (Table 2; Fig. 2A, B). At 9 d, the incidence of diapause was higher for females compared with males only when squares were fed thrice weekly (Table 2). By 12 d of age the probability of diapause was higher for females compared with males on the boll diet and when squares were fed thrice weekly, but no difference between sexes was demonstrated for weevils fed squares daily (Table 2; Fig. 2A, B). In general, the weevil sexes responded similarly to feeding regime except at the earliest age of assessment (6 d), when the incidence of diapause for males was substantially lower compared with females.

Within each diet, the occurrence of diapause for females was relatively consistent among ages (Table 2; Fig. 2A). Simple effect tests for males fed bolls, or squares thrice weekly, indicated an age effect (Table 2) but differences among ages were not demonstrated after adjustment for multiplicity (bolls, $0.05 <$ adjusted-$P < 0.99$; squares, $0.07 <$ adjusted-$P < 0.63$). In contrast, the probability of diapause increased between 6 and 9 d for males fed squares daily (adjusted-$P < 0.01$), but not between 9 and 12 d (adjusted-$P = 0.66$; Fig. 2B). Therefore, males fed squares thrice weekly exhibited a low incidence of diapause, males fed bolls exhibited a high incidence of diapause, and males fed squares daily exhibited a high incidence of diapause at ages >6 d.

The influence of diet on vitellogenesis was dependent on female age (diet × age interaction, Table 2). Simple effect tests indicated a difference among diets only at 9 d (Table 2; Fig. 2C), when oocytes with yolk or eggs were more prevalent in females fed squares thrice weekly compared with females fed squares daily (adjusted-$P = 0.01$, Fig. 2C). Incidence of vitellogenesis did not differ among ages of females fed bolls. Although the age effect was significant for weevils fed squares daily (Table 2), comparisons among ages did not indicate differences after adjustment for multiplicity ($0.05 <$ adjusted-$P < 0.91$). For females fed squares thrice weekly, the incidence of vitellogenesis was higher at 9 and 12 d compared with 6 d (adjusted-$P \leq 0.01$; Fig. 2C). Therefore, a temporal pattern of development of vitellogenic oocytes or eggs was demonstrated only for females fed squares thrice weekly.

The occurrence of oosorption was highly variable both among and within diet regimes (Fig. 2D). Consequently, no influence of diet, weevil age, or their interaction was detected (Table 2). Overall, 58% (SE = 7.4%) of females containing eggs or oocytes with yolk also exhibited oosorption.

Numbers of combined oocytes and eggs per female ($n = 5$ to 29 females per diet × age combination) were not influenced by diet ($F = 1.57$; df = 2, 44.4; $P = 0.22$), age ($F = 2.52$; df = 2, 44.8; $P = 0.09$), or their interaction ($F = 0.86$; df = 4, 42.1; $P = 0.50$). The mean (± SE) number of oocytes ranged from 5.9 (± 0.99; bolls at 9 d) to 11.3 (± 1.97; squares daily at 6 d) and the overall mean was 8.2 (± 0.47) oocytes per female.

Influence of boll size. Although none of the interactions among diet, age, or weevil sex were significant, P-values for diet × sex and age × sex interactions suggested nonnegligible effects (Table 3). Simple effect tests indicated different diapause responses among boll sizes within weevil sex (Table 3). For females, the incidences of diapause in response to 15–20- and 20–25-mm-diameter bolls were

Table 3. Analyses of incidence of diapause, vitellogenesis, and oosorption for boll weevil adults fed bolls of 10–15 (S), 15–20 (M), or 20–25-mm diameter (L) for 6, 9, or 12 d at 29.4°C with a 13:11 (L:D) h photoperiod.

	Diapause				Vitellogenesis			Oosorption		
Effect	*F*	df	*P*	Effect*	*F*	df	*P*	*F*	df	*P*
Diet	18.6	2, 27	<0.01	Diet	2.74	2, 24	0.09	4.58	2, 24	0.02
Age	1.39	2, 27	0.27	Age	4.52	2, 24	0.02	0.41	2, 24	0.67
Diet × age	0.49	4, 27	0.74	Diet × age	1.06	4, 24	0.40	1.56	4, 24	0.22
Sex	16.7	1, 27	<0.01	—	—	—	—	—	—	—
Diet × sex	2.05	2, 27	0.15	—	—	—	—	—	—	—
Age × sex	2.15	2, 27	0.14	—	—	—	—	—	—	—
Diet × age × sex	0.49	4, 27	0.74	—	—	—	—	—	—	—
Diet (♀)**	15.4	2, 27	<0.01	—	—	—	—	—	—	—
Diet (♂)**	10.0	2, 27	<0.01	—	—	—	—	—	—	—
Sex (S)**	5.81	1, 27	0.02	—	—	—	—	—	—	—
Sex (M)**	8.44	1, 27	<0.01	—	—	—	—	—	—	—
Sex (L)**	6.40	1, 27	0.02	—	—	—	—	—	—	—
Sex (6 d)**	12.4	1, 27	<0.01	—	—	—	—	—	—	—
Sex (9 d)**	4.62	1, 27	0.04	—	—	—	—	—	—	—
Sex (12 d)**	1.34	1, 27	0.26	—	—	—	—	—	—	—
Age (♀)**	1.09	2, 27	0.35	—	—	—	—	—	—	—
Age (♂)**	1.78	2, 27	0.19	—	—	—	—	—	—	—

* Analyses of yolk and oosorption incidence use the same model effects.

** Simple effect tests of a main effect within a level of an interacting effect (in parentheses).

greater compared with 10–15-mm bolls (adjusted-$P < 0.01$; Fig. 3A). For males, there was a higher incidence of diapause in response to 20–25-mm bolls compared with 10–15-mm bolls (adjusted-$P < 0.01$; Fig. 3B). Simple effect tests comparing the diapause responses of males and females were significant for each boll size class (Table 3), indicating a generally higher incidence of diapause for females compared with males (Fig. 3A, B). Tests comparing the responses of sexes within ages indicated a higher incidence of diapause for females compared with males at 6 and 9 d, but not at 12 d (Table 3; Fig. 3A, B). Simple effect tests of the age × sex interaction did not indicate an age effect for either sex (Table 3). In general, both sexes exhibited a higher incidence of diapause as boll size increased, but females tended to exhibit the diapause characters earlier compared with males.

The occurrence of vitellogenesis was not influenced by diet, and the diet × age interaction appeared negligible (Table 3). The probability of vitellogenesis tended to

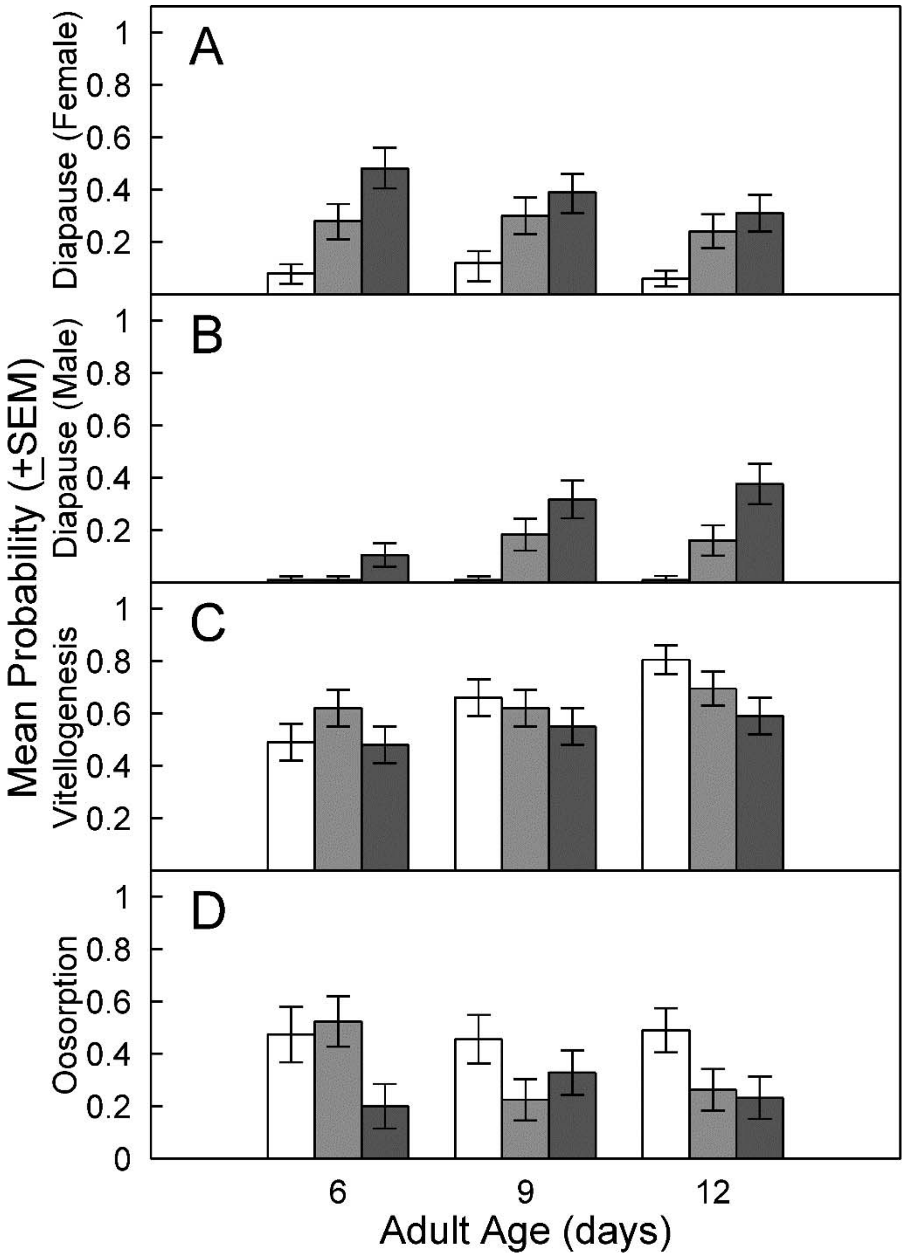

Fig. 3. Mean probability (± SE) of female diapause (A), male diapause (B), vitellogenesis (C), and oosorption (D) for boll weevils dissected at different ages after feeding as groups on cotton bolls 10–15 mm (white bars), 15–20 mm (medium gray bars), or 20–25 mm in diameter (dark gray bars) at 29.4°C and a 13:11 (L:D) h photoperiod.

increase with female age (Table 3), but pairwise comparisons indicated a significant difference only between 6 and 12 d (adjusted-$P = 0.02$; Fig. 3C).

Analyses of oosorption suggested a negligible diet × age interaction, and no effect of weevil age (Table 3), but boll size influenced the incidence of oosorption (Table 3). The probability of oosorption was higher for females fed 10–15-mm-diameter bolls compared with 20–25-mm bolls (adjusted-$P = 0.02$), whereas the incidence of oosorption for females fed 15–20-mm bolls was not different from the other boll sizes ($0.14 <$ adjusted-$P < 0.55$).

Mean (± SE) numbers of vitellogenic oocytes and eggs ($n = 24$ to 41 females per diet × age combination) varied from 9.8 (± 0.65) for 12-d-old females fed 15–20-mm-diameter bolls to 7.4 (± 0.51) for 12-d-old females fed 10–15-mm bolls. Numbers of oocytes were not influenced by female age ($F = 0.11$; df = 2, 24.1; $P = 0.90$) or diet ($F = 1.70$; df = 2, 23.9; $P = 0.22$), but the diet × age interaction appeared nonnegligible ($F = 2.56$; df = 4, 23.7; $P = 0.06$). Simple effect tests indicated differences among diets only at 12 d ($F = 4.72$; df = 2, 20.1; $P = 0.02$), when females fed 15–20-mm-diameter bolls had more oocytes and eggs compared with females fed 10–15-mm bolls (adjusted-$P = 0.02$). Females fed 15–20-mm (9.4 ± 0.37) or 20–25-mm bolls (8.4 ± 0.38) did not exhibit an age effect (15–20 mm, $F = 0.62$; df = 2, 19.5; $P = 0.55$; 20–25 mm, $F = 0.70$; df = 2, 31.9; $P = 0.50$). The age effect was significant for females fed 10–15-mm-diameter bolls ($F = 4.72$; df = 2, 20.1; $P = 0.02$). The mean number of oocytes for females fed 10–15-mm bolls was lower at 12 d compared with 6 d (9.7 ± 0.65; adjusted-$P = 0.04$), but numbers at 9 d (9.1 ± 0.63) were not different compared with other ages ($0.10 <$ adjusted-$P < 0.84$).

Discussion

Spurgeon and Suh (2017a) reported that female weevils tended to exhibit the diapause characters at an earlier age compared with males. We observed similar trends. Age dependency in the incidence of diapause was observed only for males, and was responsible for many of the interactions in our analyses. These results are explained by the morphological characters used to distinguish diapause. The ovaries of newly eclosed females are undeveloped (Spurgeon and Raulston 1998), and classification as diapausing requires only development of a hypertrophied fat body. In contrast, the testes must develop to exhibit the atrophy and opacity characteristic of diapause. In our studies, some diapausing males were not distinguishable at 6 d of age. These results support the concerns expressed by Spurgeon and Raulston (1998) regarding failure of many earlier studies to account for differences in development rates among experimental treatments.

Spurgeon and Raulston (1998) estimated that 90% of reproductive females possessed vitellogenic oocytes by 3 d at 29.4°C. Therefore, females in our studies had ample time to produce vitellogenic oocytes before the first dissection interval. Except for females fed squares thrice weekly in the second experiment, few temporal patterns of vitellogenesis were observed, and demonstrated responses were subtle. Where they occurred, trends in the incidence of vitellogenesis at 9 or 12 d of weevil age were generally opposite of trends in the incidence of diapause. This pattern was expected because the criteria of Spurgeon et al. (2003) consider vitellogenesis to signify reproduction. Absence of this pattern at 6 d of age suggests that some diapausing females had not yet developed hypertrophied fat bodies, and therefore assessment of diapause at 6 d was not as reliable as assessment at later ages.

Weevils fed squares thrice weekly (second experiment) warrant separate mention because many of them developed neither vitellogenic oocytes nor hypertrophied fat bodies. Had we used diapause criteria of some previous studies, based solely on gonadal condition (Lambremont et al. 1964, Mitchell and Taft 1966, Paula et al. 2013, Sivasupramaniam et al. 1995), the apparent diapause response of these weevils would have been higher than what we estimated. Showler (2008) reported limited reproductive development of weevils fed excised parts of squares or bolls. He concluded the morphological characters often attributed to diapause were instead consequences of deficient diets. The starvation response we observed when weevils were fed squares thrice weekly seems a more plausible explanation for the observations by Showler (2008).

Ovary development of reproductive females occurs rapidly under the temperatures we used (Spurgeon and Raulston 1998). Hilliard and Keeley (1984) observed similarly rapid development, and suggested the commitment to reproduction must occur early in adulthood. However, Grodowitz and Brewer (1987) suggested that weevils may enter diapause after committing to reproduction. A reversion from reproduction to diapause would require resorption of existing oocytes. Oosorption occurs in response to starvation (Spurgeon and Suh 2017b) or diet change (Spurgeon et al. 2003, Vanderzant et al. 1959), but there is no evidence that this response is not immediately reversible. Our results demonstrated an influence of diet on oosorption only in the third (boll size) study, when oosorption occurred more frequently, and diapause less frequently, in response to 10–15-mm-diameter bolls compared with 20–25-mm bolls. If oosorption is a symptom of a transition from reproduction to diapause, we would expect a diminished occurrence of vitellogenesis at the later ages, which was not observed. Our results did not reveal patterns to suggest a role of oosorption in diapause induction. Instead, oosorption appears a rapid response to diet quality or starvation that is separate from the diapause response. Lack of temporal patterns in vitellogenesis, oosorption, or diapause in females suggests the commitment to reproduction or diapause occurred soon after adult eclosion.

Some reports of dietary influences on diapause in the boll weevil speculate that nutrient composition is important (Keeley et al. 1977, Tingle et al. 1971). However, Hilliard and Keeley (1984) found the nutritional composition of bolls was adequate to support egg production, albeit at a lower rate compared with squares. In addition to potential influences of nutrition, behavioral aspects of host assessment are well documented. The ovipositing female prefers squares of a specific size class (about 1/3-grown) (Hilliard and Keeley 1984, Jenkins at al. 1975, McGovern et al. 1987), and some reports suggest a preference for squares that are not punctured (McGovern et al. 1987, Mitchell and Cross 1969). Greenberg et al. (2003) found the weevil could scale egg production in response to square availability even when squares were provided in excess of oviposition. Also, adult diets generally considered inadequate for egg production have been used to maintain parent laboratory colonies (Sterling and Adkisson 1966, Sterling et al. 1965, Vanderzant et al. 1959). Finally, Vanderzant and Davich (1961) found that, compared with artificial diet poured into dishes, egg production was enhanced when the same diet was formed into cylinders. Therefore, the role of behavior in host perception by the weevil appears important irrespective of any role of diet composition. Although we observed differences among weevil ages and diets in oocyte and egg numbers per female, those differences were modest and no clear trends were evident. These results suggest the diapause responses we observed were not a simple consequence of diets that were nutritionally inadequate to support egg production.

Paula et al. (2013) reported the adult diet could induce dormancy in the boll weevil, but they characterized the dormancy as quiescence (an immediate and reversible response) instead of diapause. We cannot compare their results to our findings because their criteria to distinguish dormancy were limited to gonadal conditions that were different from the criteria we used.

The dynamics of boll weevil diapause in the tropics and subtropics is a matter of controversy, in part because a portion of the weevil population in the subtropics continues reproduction so long as fruiting cotton is available (Rummel and Summy 1997). However, before the widespread adoption of modern crop termination practices, mechanized harvest, and determinant cultivars, cotton stalks in temperate production regions often remained alive in the field until they were killed by freezing. Under those conditions, fall rains often stimulated production of new squares that were used by the weevil for reproduction (Beckham 1962, Mally 1901, Reinhard 1943) under photoperiod and temperature conditions reputed to induce diapause. If diapause is primarily mediated through the weevil's perception of host or fruit maturity, instead of photoperiod and temperature, the distinctions among diapause responses of different climatic regions become less obvious. A close relationship between the weevil and host fruiting cycles is also consistent with reports of the boll weevil and other *Anthonomus* spp. on wild hosts in the tropics (Jones et al. 1989, Jones and Peruyero 2002, Lukefahr and Maxwell 1969).

Our results clearly indicate a general increase in the incidence of diapause with increasing food item maturity, except where the response was influenced by starvation. Clarification of the role of adult diet in diapause induction is necessary for meaningful investigation of additional environmental influences on diapause induction and overwinter survival. A more comprehensive understanding of diapause and its ecological implications may then be applicable to efforts to enhance management or eradication plans for intractable weevil populations in southern Texas and northern Mexico.

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