

Termination of Diapause in the Boll Weevil (Coleoptera: Curculionidae)

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Abstract

The boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), is a major pest of cotton (*Gossypium* spp. (Malvales: Malvaceae)) in Mexico, South America, and South Texas in the United States. The ability of the boll weevil to survive extended cotton-free periods has been key to its persistence as a pest despite intensive control efforts. However, the mechanism facilitating survival has been subject to debate. Whereas adult diapause has long been considered the principal survival mechanism, some authors have characterized the dormancy as a quiescence. We induced dormancy in the weevil and examined whether food type, enforced starvation, or induced flight influenced termination of the dormancy. Providing dormant adult weevils a diet favoring reproduction for 7–14 d prompted a modest termination response in female weevils and virtually no response in males. Some weevils starved ≥ 21 d resumed reproduction after exposure to a favorable diet, but most weevils remained dormant. Induced flight followed by exposure to a favorable diet prompted $>50\%$ of the weevils to terminate the dormancy. Patterns of feeding and oviposition were also useful in interpreting the termination response. These results indicate that the dormancy exhibited by the weevil is a diapause of variable intensity rather than a quiescence. A conceptual model recognizing population heterogeneity in diapause induction and intensity is consistent with reports of host-free survival and accommodates perceived differences in boll weevil ecology among temperate, subtropical, and tropical regions. This model provides a framework that will be valuable to research, management, and eradication efforts in the tropics and subtropics.

Key words. dormancy, termination, overwintering, cotton, weevil

The boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), is one of the most damaging pests of cotton (*Gossypium* spp. (Malvales: Malvaceae)) in the western hemisphere. In most cotton production systems, at least a portion of the boll weevil population is thought to enter a reproductive dormancy as the cotton crop matures (Brazzel and Hightower 1960, Carter and Phillips 1973, Graham et al. 1979) to facilitate survival of the fallow season. Whereas the reproductive dormancy in the boll weevil has been extensively studied, only recently have the cues inducing it become understood (Spurgeon and Raulston 2006; Spurgeon and Suh 2017, 2018; Spurgeon et al. 2018, 2019). However, the nature of this reproductive dormancy has been a matter of debate. Brazzel and Newsom (1959) first characterized the dormancy as a diapause, and this characterization was widely accepted. But later research

was marked by inconsistencies surrounding the factors inducing the diapause (Spurgeon and Raulston 2006). In addition, especially in the subtropics, at least a portion of the weevil population exhibits a propensity for continued reproduction so long as fruiting cotton is available (Summy et al. 1988, Rummel and Summy 1997). Although reproduction on fallow-season fruiting cotton was also frequently documented in temperate regions prior to the widespread adoption of mechanical harvest (Mally 1901, Hinds and Yothers 1909, Reinhard 1943), the consistency with which this reproduction was observed in the subtropics caused some to question the nature of the dormancy (Guerra et al. 1982, 1984; Showler 2009) or whether it occurs at all (Showler 2007, 2010).

Guerra et al. (1982) examined field-collected weevils and reported that oviposition always occurred within 2–5 d of access

to food in the laboratory. Based on the rapid initiation of reproduction, they concluded the dormancy was a quiescence instead of diapause. The initial classification of field-collected weevils as diapausing was based only on observation of the fat bodies through the dorsal abdominal cuticle (Guerra et al. 1982). Subsequently, Spurgeon et al. (2003) reported that observation through the cuticle was not a reliable method for classifying the fat body. In contrast to the report of Guerra et al. (1982), Greenberg et al. (2007) examined seasonal patterns in the preoviposition periods of trap-captured boll weevils and found the wintering weevils did not rapidly initiate oviposition when provided a diet known to promote reproduction. Paula et al. (2013) examined the responses of boll weevils to diet in Brazil and concluded the dormancy was a quiescence. Although Paula et al. (2013) reported using the classification criteria of Brazzel and Newsom (1959), their morphological criteria for distinguishing diapause were not consistent with those proposed by Brazzel and Newsom (1959) or Spurgeon et al. (2003). Whereas Paula et al. (2013) classified females as dormant based on fat body condition and lack of chorionic eggs in the ovaries, Brazzel and Newsom (1959) described nonreproductive females as lacking evidence of oogenesis. Also, Paula et al. (2013) described the testes of dormant males simply as atrophied, with or without some yellowing on their periphery, whereas Brazzel and Newsom (1959) and Spurgeon et al. (2003) indicated the testes of diapausing males were small and opaque with fat.

There are numerous definitions of diapause (Mansingh 1971, Thiele 1973, Masaki 1980, Denlinger 1986), although it is sometimes difficult to distinguish from other forms of dormancy (Andrewartha 1952, Hodek 1983). Regardless, a generally recognized distinction between diapause and quiescence lies in their termination; quiescence is terminated rapidly upon resumption of conditions favorable for development or reproduction whereas diapause is not (Mansingh 1971, Thiele 1973, Behrens 1985). The rationale of Paula et al. (2013) for concluding the dormancy was a quiescence is unclear because they did not examine its termination, and we have questioned the dormancy classifications of Guerra et al. (1982). Greenberg et al. (2007) did not examine diapause *per se*, and because the insects were field- or trap-collected, neither Guerra et al. (1982) nor Greenberg et al. (2007) knew the history of the insects they examined.

Spurgeon et al. (2003; 2008; 2018, 2019), Spurgeon and Raulston (2006), and Spurgeon and Suh (2017, 2018) have shown that the boll weevil diapause can be induced by manipulating the adult diet. In addition, Spurgeon et al. (2008, 2018), Spurgeon and Suh (2017, 2018), and Westbrook et al. (2003) have demonstrated that the laboratory-induced diapause is associated with extended host-free survival. The ability to reliably induce diapause in the laboratory provides an opportunity to examine its termination under controlled conditions and with knowledge of the history of the experimental insects. Therefore, our objective was to use diapause-induced boll weevils of known history to examine temporal patterns of termination in response to food type, starvation, and induced flight.

Materials and Methods

Our objectives were addressed in four separate experiments. The first experiment examined food-mediated diapause termination immediately following induction periods of variable duration. The second experiment used a fixed duration induction period and a postinduction feeding period of longer duration than the first experiment. The third experiment examined food-mediated diapause termination after different durations of starvation. Finally, the fourth experiment

examined the influence of induced flight on diet-mediated diapause termination.

Experimental weevils were obtained from field-collected infested flower buds (squares) of cotton near College Station, TX, during the growing season of 2001. Each collection of squares was held within a 20- × 20- × 20-cm acrylic cage ventilated with screen. Environmental conditions for rearing larvae and pupae to adulthood, and for feeding and starvation periods of adult weevils, were 29.4 ± 1°C with a 13:11 (L:D) h photoperiod. When pupae began to appear within the infested squares, they were harvested daily and placed in groups of ≤50 on a layer of moistened vermiculite within 100- × 15-mm Petri plates. The plates were examined twice daily for newly eclosed adults, which were separated by sex using the method of Sappington and Spurgeon (2000).

In each experiment, diapause was induced by providing cotton fruit (bolls, 20–25 mm diameter) at a rate of about one boll per 10 weevils. Each group of weevils was held in one of the acrylic cages described above. A water source (snap-cap vial with a cotton wick) was provided, and the bolls were replaced three times each week. During the diapause termination feeding periods, weevils were held individually within 100- × 15-mm Petri plates. Each weevil was provided a diet known to initiate and maintain reproductive development (one square, 5–9 mm diameter with intact bracteoles replaced daily, Spurgeon and Raulston 2006) and a short section of water-saturated cotton wick. Diapause status was determined by dissecting the weevils under water in a paraffin-lined Petri plate. We used the diapause criteria described by Spurgeon et al. (2003), in which females with a hypertrophied fat body and lacking vitellogenic oocytes, eggs, or follicular relics, and males with a hypertrophied fat body and small testes opaque with fat, were classed as diapausing. Weevils exhibiting any other combination of fat body and gonadal condition were classed as nondiapausing.

Experiment 1: Termination Response to Short-Term Feeding

Adults were provided the diapause-inducing diet in mixed-sex groups of 50 weevils (25 weevils of each sex). Each cage of weevils was assigned an induction period (adult ages of 3, 6, 9, or 12 d). At the end of each induction period, 10 weevils of each sex were randomly selected and diapause status was determined by dissection. Remaining weevils were provided the square diet for 7 d to promote reproductive development. Weevils entering diapause commonly cease to feed or feed only sparingly (D.W.S., unpublished data). Therefore, during the 7-d square feeding period, each square was examined for evidence of feeding as it was replaced. The boll weevil frequently samples substrates even when not feeding (Grossman 1928). To avoid confusing superficial scarring with feeding, we recorded a weevil as having fed only when the feeding puncture penetrated the floral cup of the square. The experiment was conducted three times, each time with a new cohort of weevils.

All analyses were conducted using SAS (SAS Institute 2012). We evaluated the influence of diet switching on diapause status using a conditional generalized linear mixed model with a binomial distribution and events/trials syntax (PROC GLIMMIX). Model parameters were estimated using Laplace estimation. Comparisons of diapause status between sexes at early ages can be misleading because females without ovary development merely need to accumulate fat to be classified as diapausing; males must exhibit testicular atrophy which tends to occur at later ages (Spurgeon and Suh 2017). Therefore, data for males and females were analyzed separately. Fixed effects included duration of the induction (boll feeding) period, whether the weevils were dissected at the end of the induction period or

were provided squares (fed squares), and the induction period \times fed squares interaction. Repetition of the experiment was a random effect. Where the interaction of age \times fed squares appeared non-negligible, simple effect tests were examined. Experiment-wise error was controlled using the SIMULATE option for comparisons among the treatment combinations. Means (probabilities) on the data scale were obtained using the inverse link function (ILINK option). The analysis of male diapause occurrence did not initially return reasonable parameter estimates because of complete separation (no males dissected after 3 d of boll feeding exhibited the characters of diapause). To allow estimation of the parameters for other feeding ages, the frequency of males exhibiting diapause at 3 d was rescaled from 0 to 0.5 in each experimental repetition.

The numbers of days on which the weevils fed once they were exposed to squares were examined separately for males and females in contingency tables (PROC FREQ). For some treatment combinations, all weevils fed on ≥ 3 d after being provided squares. Therefore, counts of weevils feeding for 0–3 d were pooled to avoid zero cell counts. In the contingency tables, the duration of diapause induction formed rows, the numbers of days on which feeding occurred formed columns, and repetitions formed strata which are analogous to blocks. Differences in temporal patterns of square feeding among diapause induction durations were assessed using the single-df Mantel–Haenszel nonzero correlation statistic (Q_{CS} , Stokes et al. 2012) with modified ridit scoring.

Experiment 2: Termination Response to Extended Feeding

The diapause termination response to extended access to squares was examined to determine whether the 7-d square feeding period in the preceding experiment was too short to elicit a termination response. Diapausing adults were obtained as in the previous experiment (mixed-sex groups of 50 weevils; 25 weevils of each sex) except the diapause induction period was 14 d. This induction period corresponds with greater host-free survival compared with 7- or 21-d periods (Spurgeon et al. 2018). At the end of the induction period, 10 weevils of each sex were dissected to determine diapause status, and remaining weevils were switched to the individual square diet. During the square feeding period, feeding activity of males was monitored daily as previously described. Females were monitored for oviposition, which involved daily inspection of the Petri dish and dissection of the square to detect eggs. Females provided squares were dissected when oviposition was detected, or after 14 d if they failed to oviposit. Males were dissected after feeding on squares for 14 d. The experiment was conducted four times.

The incidence of diapause was analyzed using a conditional generalized linear mixed model with a binomial distribution and events/trials syntax. Occurrence of diapause was considered an event. The model included fixed effects of weevil sex, whether they were dissected at the end of the induction period or after subsequent exposure to squares (fed squares), and their interaction. Repetition of the experiment was a random effect. A nonnegligible sex \times fed squares interaction was examined with simple effects tests.

Square feeding activity by males was examined in a contingency table with diapause status (determined by dissection) as rows, numbers of days on which the weevils fed as columns, and repetitions of the experiment as strata. The nonzero correlation statistic was used to assess association between feeding activity and diapause status as in the previous experiment.

Spurgeon and Raulston (1998) estimated the median time for female weevils to develop one or more chorionated eggs was about

3 d at 29.4°C. Therefore, females exposed to squares were pooled into three classes based on their preoviposition times: 1–3 d, 4–14 d, and not ovipositing. Females ovipositing in ≤ 3 d of exposure to squares were likely reproductive at the beginning of the square feeding period, whereas females requiring ≥ 4 d to oviposit were more likely to have terminated diapause. The binomial proportion and Agresti–Coull 95% confidence interval was calculated for each oviposition class.

Experiment 3: Termination Response to Starvation

Modern cotton production systems typically involve destruction of the standing cotton stalks following harvest. Therefore, overwintering boll weevils are generally denied access to the cotton host until the development of regrowth or volunteer plants, or planting of the following cotton crop. To examine the possible role of temporary starvation in the termination of diapause, single-sex groups of 75 weevils each were provided the diapause-inducing boll diet within two acrylic cages (one of males and one of females) previously described. At the end of the 14-d boll feeding period, 20 weevils were randomly selected from each cage for determination of diapause status. Food items in the cages were replaced with crumpled craft paper (30 \times 45 cm) to provide resting sites for the remaining weevils during the starvation period. Starvation cages were held at $23.9 \pm 1^\circ\text{C}$ with a 13:11 (L:D) h photoperiod. After each of 7 and 21 d of starvation, 10 weevils randomly selected from each cage were transferred to individual Petri plates where they were provided the reproduction-promoting square diet for 14 d. After 35 d of starvation, the weevils remaining in each cage were evenly divided between two groups. Weevils of one group were individually transferred to squares for 14 d, and weevils of the other group were immediately dissected to determine diapause status. Weevils on the square diet were monitored for feeding activity (males) and oviposition (females) as described for the second experiment. Females were dissected when oviposition was detected or at the end of the 14-d square feeding period; males were dissected after feeding on squares for 14 d. The experiment was conducted four times.

The experimental effects (sex, fed squares, and starvation duration) were not crossed so the incidence of diapause was examined in a means model (Milliken and Johnson 1984) with fixed effects of weevil sex, treatment (the combination of fed squares and starvation duration), and their interaction. A conditional analysis with a binomial distribution, events/trials syntax, and repetition of the experiment as a random effect was used. Only a single weevil (female) dissected after 35 d of starvation was not diapausing, which created a problem with complete separation. Because we used a means model, the data for this treatment (fed bolls for 14 d and starved for 35 d) were excluded from the analysis. When the probability of diapause was compared among treatments, the type-I experiment-wise error was controlled using the SIMULATE option. The patterns of oviposition (females) and feeding activity (males) of weevils provided squares were examined in contingency tables as previously described. Duration of starvation formed the table rows and preoviposition class or the numbers of days on which males fed formed the table columns.

Experiment 4: Termination Response to Flight

Dispersal of boll weevils by flight from late season cotton is well documented in both temperate and tropical production systems (Fenton and Dunnam 1928, Guerra 1986, Jones et al. 1992, Moody et al. 1993). These flights are either by reproductive weevils in search of oviposition sites, or are relatively short-distance flights by

diapausing weevils in search of overwintering habitat (Moody et al. 1993, Raulston et al. 1996). To examine the potential influence of short-duration flight on diapause termination, single-sex groups of 50 weevils (one group of each sex) were fed the diapause-inducing diet for 14 d within the previously described cages. At the end of the diapause induction period, the weevils within each cage were divided into four groups: 1) dissected to provide an initial estimate of diapause ($n = 10$); 2) starved for 2 d and then dissected ($n = 15$); 3) starved and flown on 2 d and then dissected ($n = 10$); and 4) starved and flown on 2 d then provided the reproduction-promoting square diet for 14 d (males) or until oviposition was observed (females) and dissected ($n = 15$). The second and third treatments were included to ensure any immediate starvation- or flight-induced change in fat body condition was detected. During the 2-d starvation period, the weevils in the second treatment (starved, not flown) were held within a starvation cage as described for the third experiment except the temperature was $29.4 \pm 1^\circ\text{C}$.

Weevils assigned to flight treatments (third and fourth treatments) were flown on stationary tethers. A rectangular frame of 0.6-cm-thick acrylic sheeting (approximately $30 \times 10 \times 25$ cm [width by depth by height]) was constructed to hold five removable acrylic shelves (approximately 29×10 cm [width by depth]). Five evenly spaced holes were drilled in the 0.6-cm face of each shelf to accommodate a short (≈ 2.5 cm) piece of plastic tubing with an inner diameter of 0.5 mm. Tethers for the weevils were constructed from nylon monofilament fishing line (0.48 mm diameter, about 1 cm long) so that one end of the completed tether fit snugly within the plastic tubing. The exposed end of the tether was flattened with pliers and trimmed to a point to provide a surface for attachment to the dorsum of the prothorax of the weevil. Each tether was attached to a weevil using cyanoacrylate adhesive so that the curvature of the monofilament extended forward and upward from the weevil.

Tethered weevils were flown for a 2-h period (1000–1200 h) on each of two consecutive days except for females of the first experimental repetition, which were flown for 4 h on the first day. At the beginning of each flight period and at 30-min intervals thereafter, each shelf was lifted from the frame and moved through an arc (≈ 1 m) three times to induce tethered weevils to fly. At the conclusion of the first flight period, the weevils were individually placed in 1.5-ml microcentrifuge tubes. Each tube was ventilated by a 1-mm diameter hole in the conical end. The tubes containing weevils were held in the same environmental chamber as the starved weevils that were not flown. Because the tethers were easily dislodged, storage in the microcentrifuge tubes minimized the number of weevils requiring reattachment of the tether for the second flight period. At the end of the second flight period, the tethers were removed without injury to the weevils, and the weevils were either dissected to assess diapause status, or they were provided the square diet for 14 d to terminate diapause. Both flight periods were monitored using a video

camera. From these recordings, we determined the number of flights ≥ 5 s and the total cumulative flight time for each weevil. Oviposition (females) and feeding activity (males) of weevils provided squares after flight were monitored as previously described. The experiment was conducted four times.

The probability of diapause was examined using a conditional means model with a binomial distribution as previously described. Fixed effects were treatment combination (fed bolls; fed bolls and starved; fed bolls, starved, and flown; fed bolls, starved, flown, and fed squares), weevil sex, and their interaction. Repetition of the experiment was a random effect. Pairwise comparisons among the treatments were adjusted for multiplicity using the SIMULATE option.

The relationship between diapause status and number of flights per hour was examined for weevils that were dissected immediately after the second flight period. We used the number of flights per hour as the response instead of total flights in order to accommodate the longer first flight period for females of the first experimental repetition. A Gaussian distribution was used with fixed effects of weevil sex, diapause status, and their interaction, and a random effect of experimental repetition. Residual plots indicated heterogeneity of variance that was addressed by allowing different variances for the two sexes. Degrees of freedom were adjusted with the Kenward–Roger option. Total flight time per hour was similarly examined except the model accommodated heterogeneous variances between the diapause classes (diapausing and nondiapausing). Nonnegligible interactions were examined using simple effect tests.

Numbers of flights per hour and total flight time per hour were also examined for weevils that were fed squares after flight. The models were the same as those for weevils dissected immediately after flight, except the residuals did not indicate heterogeneous variances. In addition, feeding activity of males and oviposition by females were examined in contingency tables as previously described. Because the numbers of males feeding ≤ 9 d were small, table columns representing weevils that fed on 0–9 d were collapsed into a single column. Diapause status then formed rows in the contingency table, which was stratified by experimental repetition. The pattern of oviposition was examined exactly as previously described, including use of the preoviposition classes of 1–3 d, 4–14 d, and not ovipositing.

Results

Experiment 1: Termination Response to Short-Term Feeding

The estimated probability of diapause for females dissected after feeding on the boll diet for 3 d tended to be lower compared with longer feeding periods (Table 1; Fig. 1a). In addition, females provided squares for 7 d after the boll feeding periods exhibited diapause less often than females that were dissected immediately after

Table 1. Tests of model effects on the probability of diapause for adult boll weevils fed cotton bolls for different durations and then either dissected immediately, or after feeding on squares for 7 d at $29.4 \pm 1^\circ\text{C}$ with a photoperiod of 13:11 (L:D) h

Model effect	Females			Males		
	F	df	P	F	df	P
Age ^a	3.94	3, 14	0.031	10.19	3, 14	<0.001
Fed squares	22.40	1, 14	<0.001	6.10	1, 14	0.027
Age \times Fed squares	0.19	3, 14	0.900	2.91	3, 14	0.071

The analysis used a conditional model with a binomial distribution.

^aWeevil adult ages were 3, 6, 9, and 12 d when dissected or switched from the boll to the square diet.

the induction period regardless of its duration (Table 1; Fig. 1a). The negligible interaction between boll feeding period and whether females were dissected or fed squares (Table 1) indicated the response to square availability was similar among the durations of boll feeding. However, reductions in the incidence of diapause in response to square feeding were relatively small, and the incidence of diapause after square feeding remained high. This suggests the observed decrease in the incidence of diapause for female weevils fed squares may represent failure to complete the diapause program rather than termination of diapause.

In contrast to females, the interaction between boll feeding duration and whether males were fed squares appeared nonnegligible (Table 1). Simple effect tests indicated a significant effect of boll feeding duration whether male weevils were dissected immediately after boll feeding ($F = 6.70$; $df = 3, 4$; $P = 0.005$) or after feeding on squares ($F = 4.22$; $df = 3, 14$; $P = 0.025$). For weevils dissected immediately after the induction period, the probability of diapause was lower after feeding on bolls for 3 d compared with all other ages (Fig. 1b). A similar, but less distinct pattern was observed for weevils provided squares after the boll feeding period (Fig. 1b). Also, the incidence of diapause for weevils fed bolls for 3 d and then fed squares for 7 d was higher than for weevils dissected after feeding on bolls for 3 d (Fig. 1b). These patterns suggest that dissections of 3-d-old males underestimated the incidence of diapause, and that males

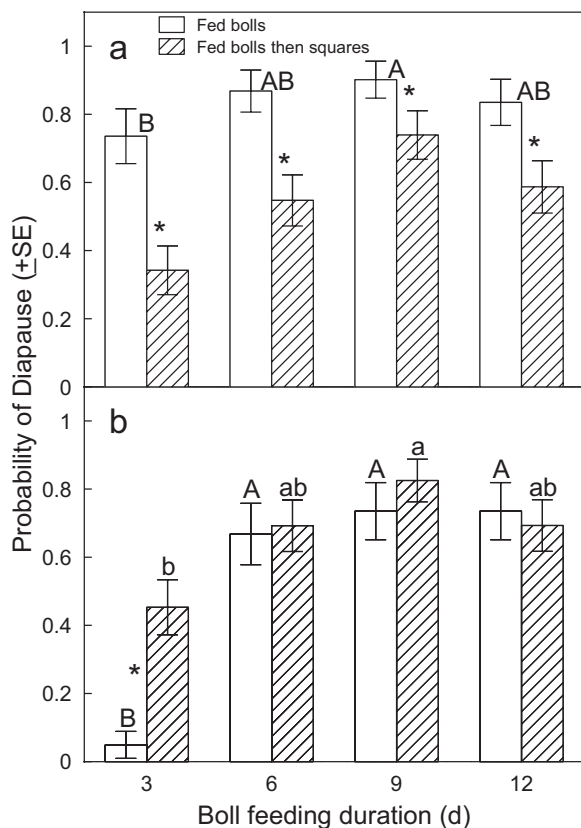


Fig. 1. Mean probability (\pm SE) of diapause for boll weevil adults fed cotton bolls for 3–12 d at 29.4°C with a 13:11 (L:D) h photoperiod and then dissected (fed bolls) or fed squares for an additional 7 d before dissection (fed bolls then squares). (a) Females, bars indicating duration of the boll feeding period with the same upper case letter are not significantly different (adjusted $P > 0.05$). (b) Males, bars indicating duration of the boll feeding period with the same upper case letter (fed bolls) or lower case letter (fed bolls then squares) are not significantly different (adjusted $P > 0.05$). Asterisk (*) indicates a significant difference between weevils fed bolls or fed bolls and then squares ($\alpha = 0.05$).

initially fed bolls tended to complete the diapause program when switched to squares rather than terminating diapause.

Examinations of feeding patterns indicated a significant association between the numbers of days on which weevils fed on squares and duration of the prior boll feeding period for both females ($Q_{CS} = 38.066$, $df = 1$, $P < 0.001$) and males ($Q_{CS} = 46.140$, $df = 1$, $P < 0.001$). Patterns in feeding activity for females and males were roughly similar regardless of the boll feeding duration (Fig. 2a–d). For both sexes, the numbers of days on which the weevils fed on squares tended to decline with increased duration of the preceding boll feeding period. This suggests a decline in feeding activity with an increase in the incidence of diapause, which is consistent with our previous, unpublished observations. Overall, our results failed to unambiguously demonstrate immediate termination of diapause in response to improved conditions (diet).

Experiment 2: Termination Response to Extended Feeding

Analysis of the probability of diapause occurrence for adult weevils fed bolls for 14 d indicated a nonnegligible interaction between weevil sex and whether weevils were dissected immediately after boll feeding or were fed squares for an additional 14 d (Table 2). Simple effect tests within feeding treatment did not indicate differences between sexes in the probability of diapause whether diapause was assessed after boll feeding ($F = 3.26$; $df = 1, 9$; $P = 0.104$) or after feeding on squares ($F = 1.57$; $df = 1, 9$; $P = 0.242$). Simple effect tests within weevil sexes indicated no effect of postinduction square feeding on males ($F = 0.01$; $df = 1, 9$; $P = 0.935$; Fig. 3). In contrast,

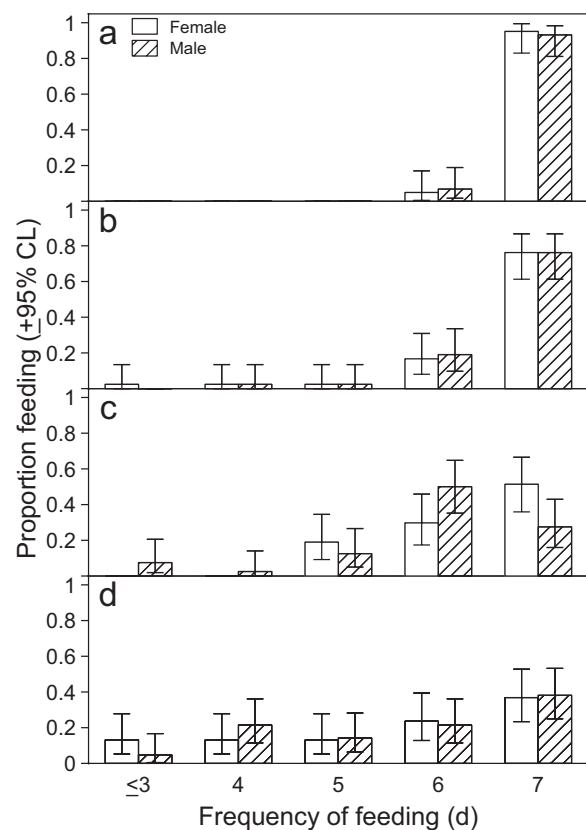


Fig. 2. Frequency distributions (proportion \pm 95% CL) of the numbers of days adult boll weevils fed when provided to cotton squares for 7 d after being fed on bolls at 29.4°C with a 13:11 (L:D) h photoperiod for (a) 3, (b) 6, (c) 9, or (d) 12 d.

Table 2. Tests of model effects on the probability of diapause for adult boll weevils fed cotton bolls for 14 d and then either dissected immediately or after feeding on squares for 14 d at $29.4 \pm 1^\circ\text{C}$ with a photoperiod of 13:11 (L:D) h

Model effect	<i>F</i>	df	<i>P</i>
Sex	0.78	1, 9	0.401
Fed squares	4.38	1, 9	0.066
Sex \times Fed squares	4.81	1, 9	0.056

The analysis used a conditional model with a binomial distribution.

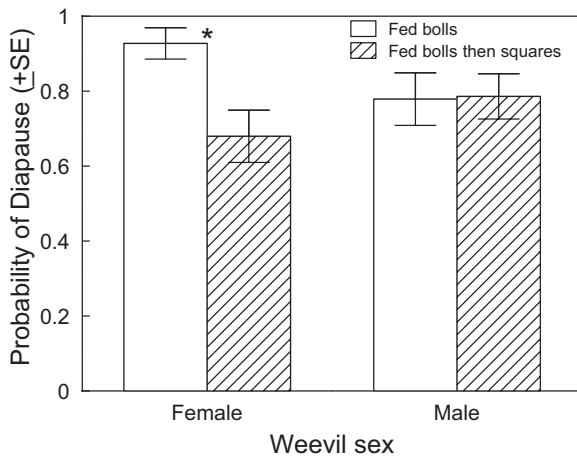


Fig. 3. Mean probability (\pm SE) of diapause for adult boll weevils fed cotton bolls for 14 d at 29.4°C with a 13:11 (L:D) h photoperiod and then dissected (fed bolls) or fed squares for 14 d before dissection (fed bolls then squares). The asterisk (*) indicates a significant difference between female weevils fed bolls and those fed bolls and then squares ($P = 0.025$).

feeding on squares reduced the incidence of diapause in females ($F = 7.19$; $df = 1, 9$; $P = 0.025$; Fig. 3), although this reduction was modest. These results were consistent with those of the first experiment, where females exhibited a limited but measurable termination response to square feeding, whereas males were unresponsive.

Examination of oviposition patterns suggested about 11% (95% confidence limits (CL), 5–22%) of females were likely reproductive at the end of the boll feeding diapause induction period because they oviposited within 3 d of exposure to squares. However, the majority of females (70%; 95% CL, 57–80%) did not oviposit during the 14-d square feeding period, and all of these females were classed as diapausing by dissection. Therefore, only about 20% (95% CL, 11–32%) of females exposed to squares after the induction period exhibited a diapause termination response.

Examination of male feeding patterns indicated a significant association between the numbers of days the males fed and their diapause status ($Q_{CS} = 4.38$, $df = 1$, $P = 0.037$; Fig. 4). The observed patterns of feeding were generally consistent with those observed in the first study in that males remaining in diapause at the end of the square feeding period tended to feed less often compared with males that were reproductive.

Experiment 3: Termination Response to Starvation

Analyses of diapause incidence for weevils fed bolls for 14 d and then starved for different intervals before being fed squares suggested a negligible interaction between weevil sex and duration of starvation (Table 3). The probability of diapause tended to be higher for males compared with females regardless of the duration

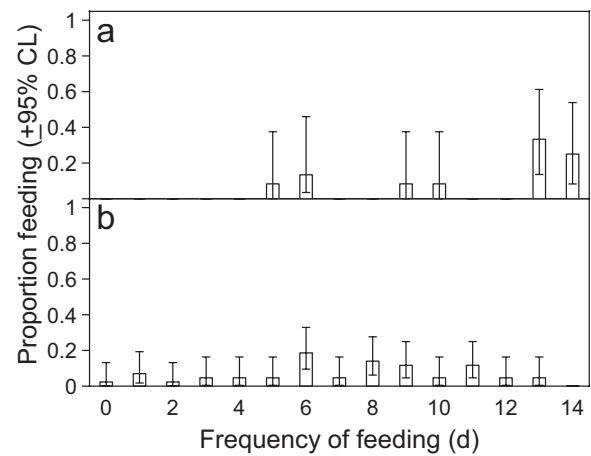


Fig. 4. Frequency distributions (proportion \pm 95% CL) of the numbers of days (a) nondiapausing and (b) diapausing male boll weevils fed on cotton squares during a 14-d feeding period after being fed bolls for 14 d at 29.4°C with a 13:11 (L:D) h photoperiod. Diapause status was determined by dissection at the end of the square feeding period.

Table 3. Tests of model effects on the probability of diapause for adult boll weevils fed cotton bolls for 14 d and then dissected immediately, or after starvation (7, 21, or 35 d) followed by access to cotton squares for 14 d at $29.4 \pm 1^\circ\text{C}$ with a photoperiod of 13:11 (L:D) h

Model effect	<i>F</i>	df	<i>P</i>
Sex	8.89	1, 21	0.007
Treatment ^a	10.79	3, 21	<0.001
Sex \times Treatment	1.55	3, 21	0.231

The analysis used a conditional, means model with a binomial distribution.

^aTreatments were 1) fed bolls for 14 d, 2) fed bolls for 14 d, starved for 7 d, and fed squares for 14 d, 3) fed bolls for 14 d, starved for 21 d, and fed squares for 14 d, and 4) fed bolls for 14 d, starved for 35 d, and fed squares for 14 d.

of starvation (Table 3; Fig. 5). For both weevil sexes, the probability of diapause tended to decrease with increased duration of starvation compared with weevils that were dissected immediately after the boll feeding period (Table 3; $P < 0.001$; Fig. 5). This trend was especially evident for weevils that were starved ≥ 21 d before they were provided squares. However, the termination response was limited even for weevils starved for 35 d before they were provided squares. In contrast, all of the male weevils ($n = 56$) and 98% of the female weevils ($n = 43$) that were dissected immediately after the 35-d starvation period (excluded from statistical analysis) were classed as diapausing. This suggested that the modest termination response observed for the other weevils was caused by exposure to squares.

When weevils were provided squares after the various starvation periods, the oviposition patterns for females ($Q_{CS} = 0.138$, $df = 1$, $P = 0.710$; Fig. 6) and the feeding patterns for males ($Q_{CS} = 0.368$, $df = 1$, $P = 0.544$; Fig. 7) were similar among the different durations of starvation. Results of these analyses were consistent with the analyses of diapause incidence, which suggested modest differences in the termination responses to the different starvation durations.

Experiment 4: Termination Response to Flight

The probability of diapause was lower for weevils fed squares for 14 d after induced flight compared with the other treatments (Table 4, Fig. 8). The probability of diapause was similar among

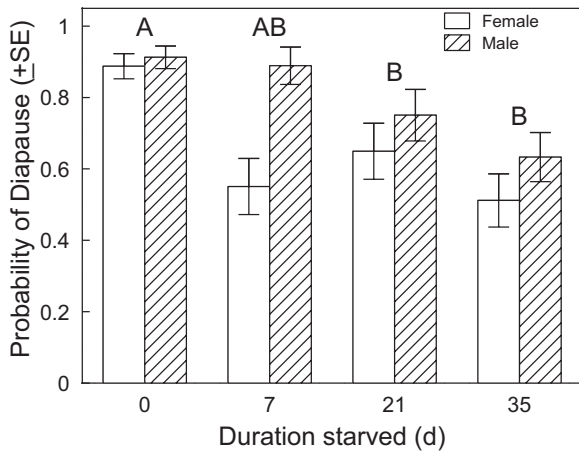


Fig. 5. Mean probability (\pm SE) of diapause for adult boll weevils fed cotton bolls for 14 d at 29.4°C with a 13:11 (L:D) h photoperiod and then dissected immediately (duration starved = 0 d), or after starvation periods of 7, 21, or 35 d followed by exposure to squares for 14 d. Probabilities of diapause for durations of starvation labeled with the same upper case letter are not significantly different (adjusted $P > 0.05$).

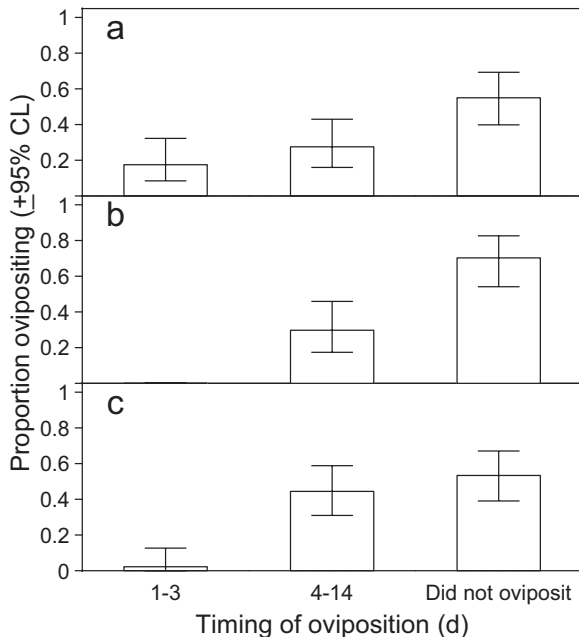


Fig. 6. Temporal pattern of first oviposition (proportion \pm 95% CL) for adult female boll weevils fed bolls for 14 d at 29.4°C with a 13:11 (L:D) h photoperiod and then provided squares for 14 d after starvation periods of (a) 7, (b) 21, or (c) 35 d.

weevils dissected immediately after the 14-d boll feeding period, after boll feeding followed by 2 d of starvation, and after boll feeding followed by starvation and two daily bouts of induced flight (Table 4, Fig. 8). The incidence of diapause was also generally higher for females compared with males, and the weevil sex by flight treatment interaction appeared negligible (Table 4). These results indicated that starvation for 2 d, or starvation and flight on 2 d, did not produce morphological responses, such as depletion of the fat body, which could be mistaken for termination of diapause. The extent of diapause termination in response to flight was relatively high, with approximately half of the diapausing weevils initiating reproductive development after feeding on squares. Although a substantial proportion of the weevils remained in diapause following flight and

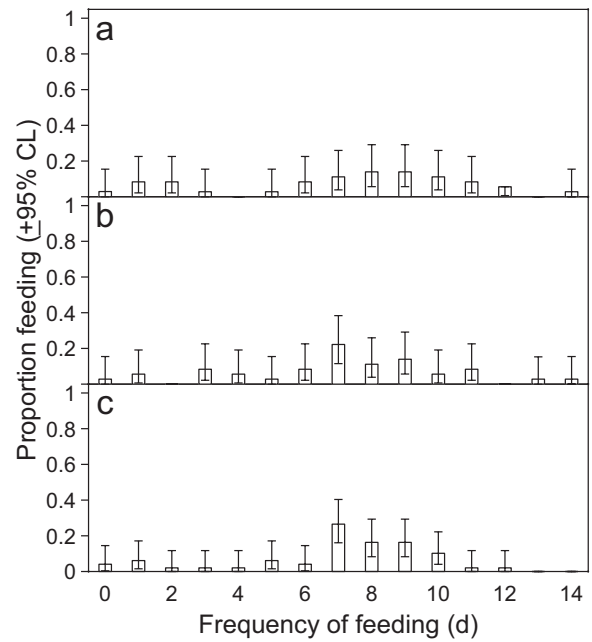


Fig. 7. Frequency distributions (proportion \pm 95% CL) of the numbers of days male boll weevils fed on squares during a 14-d feeding period after being fed bolls for 14 d at 29.4°C with a 13:11 (L:D) h photoperiod and then starved for (a) 7, (b) 21, or (c) 35 d.

Table 4. Tests of model effects on the probability of diapause for adult boll weevils fed cotton bolls for 14 d and dissected immediately, or dissected after 2 d of starvation, 2 d of starvation and induced flight, or 2 d of starvation and induced flight followed by access to squares for 14 d at 29.4 \pm 1°C with a photoperiod of 13:11 (L:D) h

Model effect	F	df	P
Sex	18.30	1, 21	<0.001
Treatment ^a	15.22	3, 21	<0.001
Sex \times Treatment	1.73	3, 21	0.191

The analysis used a conditional, means model with a binomial distribution.

^aTreatments were 1) fed bolls for 14 d, 2) fed bolls for 14 d and starved for 2 d, 3) fed bolls for 14 d, starved for 2 d, and induced to fly on 2 d, and 4) fed bolls for 14 d, starved for 2 d, induced to fly on 2 d, and fed squares for 14 d.

then square feeding, the termination response to this combination of factors was more pronounced than the responses observed to other factors.

Analyses of the numbers of flights per hour for weevils dissected after the second day of flight did not indicate differences between weevil sexes or diapause status, and the sex by diapause status interaction appeared negligible (Table 5). Females exhibited an average (\pm SE) of 2.2 (\pm 0.42) flights per hour compared with 3.7 (\pm 0.72) flights per hour for males. Weevils that were reproductive during the flight periods flew an average of 3.0 (\pm 0.65) times per hour whereas weevils that were in diapause took 2.9 (\pm 0.39) flights per hour. For weevils fed squares for 14 d after flight, numbers of flights were similar between weevil sexes, and between diapausing and nondiapausing weevils. The sex by diapause status effect was also negligible (Table 5). These females averaged 2.4 (\pm 0.34) flights per hour compared with 2.8 (\pm 0.36) flights per hour for males, and reproductive weevils flew 2.4 (\pm 0.33) times per hour compared with 2.8 (\pm 0.37) flights per hour for diapausing weevils. These results indicated that the propensity to fly was not indicative of diapause or its termination.

In contrast to the numbers of flights, cumulative durations of flight per hour were related to both weevil sex and diapause status. Whether diapause status was assessed immediately after the second flight period, or after flight and feeding on squares, the sex by diapause status interaction was significant (Table 6). For weevils dissected immediately after the second flight period, simple effect tests within weevil sex did not indicate differences between diapause and reproductive weevils for either males ($F = 0.01$; $df = 1, 3.23$; $P = 0.923$) or females ($F = 5.50$; $df = 1, 3.75$; $P = 0.083$), although results suggested a tendency for increased flight time for reproductive females (Fig. 9a). Also, there were no differences in duration of flight per hour between female and male diapausing weevils ($F = 1.26$; $df = 1, 70.03$; $P = 0.265$). However, reproductive female weevils flew longer than reproductive males ($F = 8.38$; $df = 1, 54.98$; $P = 0.005$; Fig. 9a). When diapause status was determined after flight and square feeding, the overall pattern in cumulative flight time per hour appeared similar to that for weevils assessed immediately after flight (Fig. 9b), although the statistical separations were different. For weevils fed squares after flight, the simple effect tests indicated longer flight durations for nondiapausing females compared with females that remained in diapause ($F = 9.42$; $df = 1, 94.47$; $P = 0.003$), but no difference in the flight times of nondiapausing and diapausing males ($F = 0.05$; $df = 1, 101$; $P = 0.831$; Fig. 9b). Simple effect tests of weevil

sex within diapause status did not indicate differences between sexes in cumulative flight time per hour for diapausing ($F = 0.94$; $df = 1, 98.24$; $P = 0.336$) or nondiapausing weevils ($F = 3.91$; $df = 1, 100.6$; $P = 0.051$), although the latter test was suggestive of greater cumulative flight duration per hour for nondiapausing females compared with males (Fig. 9b). Trends in flight time per hour among combinations of weevil sex and diapause status determined immediately after flight were similar to those for weevils assessed after square feeding (Fig. 9a and b). Male flight durations were uninformative because no difference was found between diapausing and nondiapausing males irrespective of when diapause was assessed. In contrast, nondiapausing females tended to exhibit greater cumulative flight times compared with diapausing females, regardless of whether diapause was assessed immediately after flight or after flight and square feeding. However, the mean total flight duration per hour was considerably higher for females dissected immediately after flight compared with females fed squares after flight. This is because many of the females classed as nondiapausing after flight and square feeding had terminated diapause. That is, they exhibited relatively short flight durations because they were in diapause during the flight periods.

The temporal pattern of first oviposition for females flown and then fed squares differed between nondiapausing and diapausing females ($Q_{CS} = 39.176$, $df = 1$, $P < 0.001$), and reflected the substantial diapause termination response indicated by the dissections. Only 11% (95% CL, 5–21%) of the weevils oviposited in the first 3 d of square exposure, which was similar to the proportions of nondiapausing females estimated by dissection after boll feeding, starvation, or starvation and flight (Fig. 8). About half of the remaining weevils (44%; 95% CL, 32–57%) oviposited after 4–14 d of exposure to squares, and about half (46%; 95% CL, 33–58%) failed to oviposit at all. Of the weevils that failed to oviposit, only one was classified as nondiapausing. Therefore, the pattern in oviposition during the square feeding period provided information regarding diapause termination that was similar to the information provided by dissection (Fig. 8). Analyses also indicated differences in the patterns of square feeding between diapausing and nondiapausing males ($Q_{CS} = 10.37$, $df = 1$, $P = 0.001$). More than 70% of the males that remained in diapause after the 14-d square feeding period fed on ≤ 9 d, whereas more than 77% of males that were reproductive after the square feeding period fed on ≥ 10 d (Fig. 10). The limited feeding by diapausing males was consistent with the feeding patterns observed in the other studies.

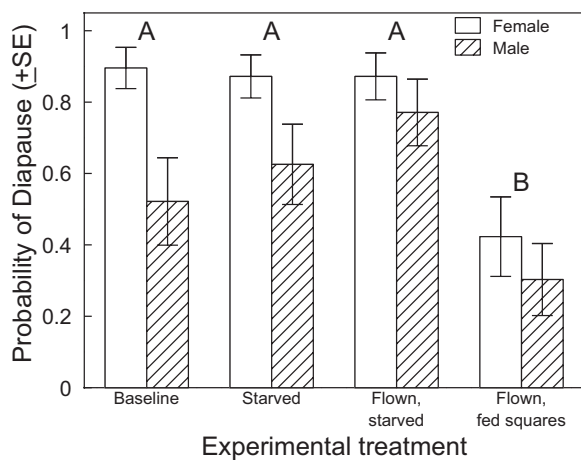


Fig. 8. Mean probability (\pm SE) of diapause among combinations of boll weevil sex and feeding treatment where weevils at 29.4°C with a 13:11 (L:D) h photoperiod were fed bolls for 14 d and then 1) dissected (baseline), 2) starved for 2 d and dissected (starved), 3) starved for 2 d and flown on 2 d (flown, starved), or 4) starved for 2 d and flown on 2 d then provided squares for 14 d before dissection (flown, fed squares). The probability of diapause was higher for females compared with males ($P < 0.001$). Feeding treatments designated by the same upper case letter are not different (adjusted $P > 0.05$).

Discussion

Overall, efforts to terminate diapause in the boll weevil yielded modest responses except when induced flight was followed by a

Table 5. Tests of model effects on the number of flights per hour by reproductive and diapausing adult boll weevils induced to fly on tethers on 2 d following a 14-d feeding period on cotton bolls at 29.4 \pm 1°C with a photoperiod of 13:11 (L:D) h

Model effect	After flight ^{a,b}			After flight and square feeding ^b		
	F	df	P	F	df	P
Sex	3.23	1, 5.78	0.124	2.46	1, 99.22	0.120
Diapause status	0.04	1, 75.14	0.847	1.47	1, 100.5	0.228
Sex \times Diapause	1.61	1, 75.14	0.208	1.24	1, 98.75	0.268

Analyses used Kenward–Roger corrected denominator df.

^aThe analysis used a heterogeneous variances model.

^bDiapause status was determined by dissection immediately after the second flight period (after flight) or after the second flight period followed by 14 d of access to squares (after flight and square feeding).

reproduction-promoting diet. Even a substantial starvation period did not prompt termination of the dormancy in a majority of the weevils. These results confirm concerns regarding the reports by Guerra et al. (1982) and Paula et al. (2013), and indicate the dormancy exhibited by the boll weevil is a diapause rather than a quiescence. These results also contradict the opinions expressed by Showler (2007, 2009, 2010) regarding the nature and occurrence of the boll weevil adult dormancy.

In addition to the morphological characters of diapause, patterns of oviposition by female weevils that were provided squares

were useful in detecting and interpreting the diapause termination response. Diapause termination indicated by the age-dependent patterns of oviposition were generally consistent with estimates of termination obtained by dissection. Differences in the temporal patterns of feeding between diapausing and nondiapausing male weevils were also consistent with estimates of diapause termination by dissection, but feeding activity was more variable and difficult to interpret compared with oviposition. Regardless, either of these responses, oviposition or feeding, may be useful to future investigators as nondestructive indicators of diapause or its termination.

Except when experimental treatments involved flight, female weevils tended to exhibit a more pronounced diapause termination response compared with males. Especially when the induction period was short, as in the first experiment, females exhibited a significant, but modest, termination response, whereas male tended to complete the diapause program even when provided a reproduction-promoting diet. Either response, but especially the response of the males, indicated the diet-mediated cues that induce diapause are received shortly after adult eclosion. Contrary to the opinions of Showler (2008), it is also apparent that the diapause responses we observed were not mere consequences of poor nutrition because diapause in most weevils was maintained or completed on diets known to promote reproduction.

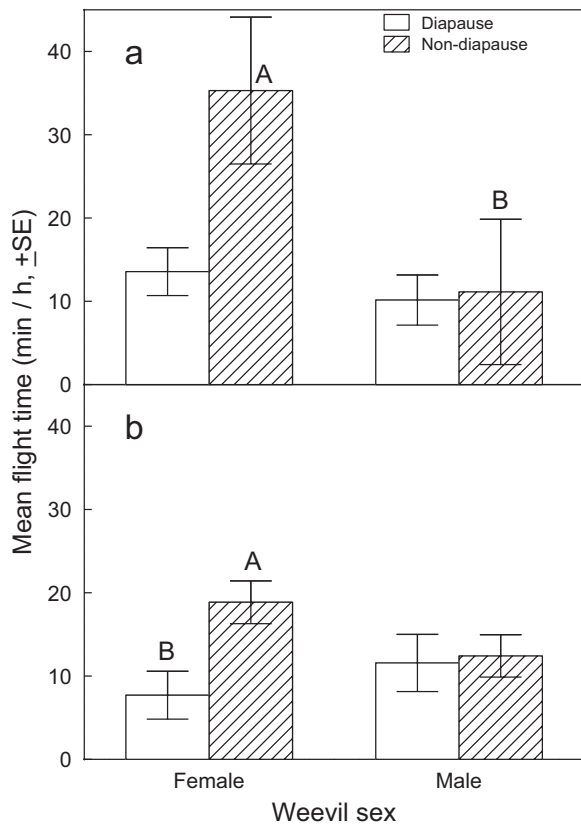


Fig. 9. Mean (\pm SE) cumulative duration of flight per hour on two consecutive days for combinations of boll weevil sex and diapause status after adult weevils were fed bolls for 14 d at 29.4°C with a 13:11 (L:D) h photoperiod. Diapause status was determined by dissection immediately after the last flight period (a) or after a 14-d postflight square feeding period (b). Hatched bars (a) or bars for females (b) marked with a different upper case letter are significantly different ($\alpha = 0.05$).

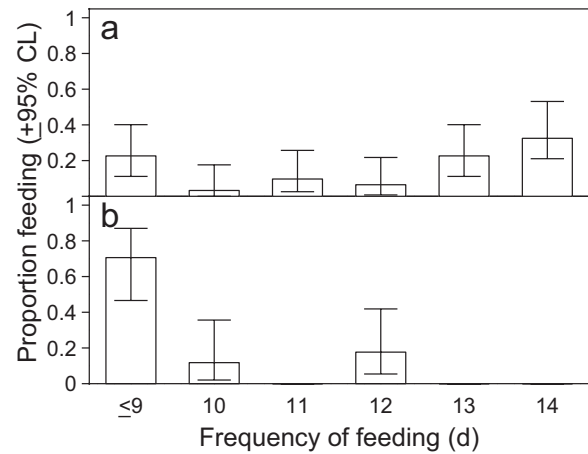


Fig. 10. Frequency distributions (proportion \pm 95% CL) of the numbers of days on which (a) nondiapausing and (b) diapausing male boll weevils fed on squares during a 14-d feeding period. Weevils were fed bolls for 14 d at 29.4°C with a 13:11 (L:D) h photoperiod, and then starved and flown on 2 d before squares were provided. Diapause status was determined by dissection at the end of the square feeding period.

Table 6. Tests of model effects on total flight duration per hour by reproductive and diapausing adult boll weevils induced to fly on tethers on 2 d following a 14-d feeding period on cotton bolls at 29.4 \pm 1°C with a photoperiod of 13:11 (L:D) h

Model effect	After flight ^{a,b}			After flight and square feeding ^b		
	F	df	P	F	df	P
Sex	9.64	1, 60.38	0.003	0.25	1, 99.79	0.620
Diapause status	1.96	1, 2.35	0.279	4.60	1, 88.33	0.035
Sex \times Diapause	5.46	1, 60.38	0.023	4.01	1, 98.93	0.048

Analyses used Kenward–Roger corrected denominator df.

^aThe analysis used a heterogeneous variances model.

^bDiapause status was determined by dissection immediately after the second flight period (after flight) or after the second flight period followed by 14 d of access to squares (after flight and square feeding).

The reduced termination responses of males compared with females may be related to respective differences in risks associated with reproduction in the presence of conflicting host cues. It is known that many female boll weevils overwinter mated and can utilize acceptable hosts without remating (Brazzel and Newsom 1959, Beckham 1962, Walker and Pickens 1962). In contrast, males play an important role in host finding through release of an aggregation pheromone that attracts both weevil sexes (Walker and Bottrell 1970, White and Rummel 1978). For a female receiving cues indicating marginal but improving host quality, the ecological risk of potentially limited reproduction on a suboptimal host might be offset by the risk of mortality in the event that a more suitable host cannot be located. The risks and benefits would be somewhat different for males, especially if they can mate prior to diapause. In that case, the potential host-finding contribution of males to successful overwintering on a population scale seems greater if the diapause program is continued, compared with termination in response to a marginal host.

Induced flight promoted termination of diapause in many of the weevils, regardless of sex. Its influence, especially for males, may indicate that flight acts as a behavioral primer, not terminating diapause itself, but facilitating diapause termination in response to location of an adequate host. In that case, the role of flight as a behavioral primer may enforce dispersal from a deteriorating host. Enforced dispersal would appear to maximize the likelihood of locating a different, possibly better host, whether that host is located during the current or a subsequent host fruiting cycle.

The primary mechanism used by the boll weevil to survive the noncotton season appears to be an adult diapause induced by impending host maturity (Spurgeon et al. 2003; Spurgeon and Raulston 2006; Spurgeon and Suh 2017, 2018; Spurgeon et al. 2018, 2019), although the specific host characters leading to diapause induction are not known. This conclusion is consistent with the ecology of the weevil, which is thought to have originated in the tropics, perhaps on wild Malvaceae (*Hampea* spp.) (Fryxell and Lukefahr 1967). Whether the boll weevil originated on *Hampea* spp., wild cottons (*Gossypium* spp.) or other wild Malvaceous hosts, the fruiting patterns of those hosts tend to be cyclic and associated with a rainy season, the timing of which varies among locations and host species (Lukefahr and Martin 1962, Lukefahr and Maxwell 1969, Burke and Clark 1976, Jones et al. 1989, Jones and Peruyero 2002). Considering the relatively small annual change in day length in the tropics compared with the temperate zone, and the asynchrony in timing of the rainy season among tropical locations, photoperiod would seem an imprecise cue for timing diapause compared with host reproductive phenology.

Conceptually, it may be useful to view the overall survival strategy of the boll weevil as a series of branch points to which variable proportions of the population respond. This variable response results in what is functionally a bet-hedging strategy. This study and others using diet-based diapause induction (Spurgeon et al. 2003; Spurgeon and Raulston 2006; Spurgeon and Suh 2017, 2018; Spurgeon et al. 2018) have indicated that the diapause response is not uniform. This nonuniformity likely reflects inter-individual heterogeneity in the propensity to diapause, which is a common characteristic of insect populations (Tauber and Tauber 1976). Therefore, a newly eclosed adult weevil receives cues indicating host quality and commits to either reproduction or diapause. The respective proportions of the population committing to reproduction or diapause are influenced by availability of undamaged fruiting forms of appropriate maturity (Spurgeon and Esquivel 2000). Such host-mediated diapause induction is not uncommon among tropical insects (Denlinger 1986).

Within the population committing to diapause, there apparently exists a range of diapause intensity, where intensity is characterized by the duration of diapause, or the ease with which it is terminated (Andrewartha 1952, Mansingh 1971, Tauber and Tauber 1976). Such range in diapause intensity is evident from the termination responses to different durations of starvation in this study and from the continuous nature of host-free survival functions typical of survival studies (Spurgeon 2008; Spurgeon et al. 2008; Spurgeon and Suh 2017, 2018; Spurgeon et al. 2018). Further, our results show that the intensity of diapause can be modified by flight in a substantial portion of the diapausing population. Weevils exhibiting the most intense diapause, that is those with the longest host-free survival, are those most likely to infest hosts during the subsequent seasonal fruiting cycle. Such a bet-hedging strategy is consistent with reports of population seasonality of tropical weevils on wild hosts (Brazzel and Newsom 1959, Lukefahr and Maxwell 1969, Jones et al. 1989, Jones et al. 1992) and with reproduction on postharvest cotton whether in the tropics (Ramalho and Wanderley 1996), subtropics (Summy et al. 1988), or temperate zone (Mally 1901, Hinds and Yothers 1909, Reinhard 1943). Therefore, this conceptual model can accommodate the perceived ecological inconsistencies among temperate, tropical, and subtropical weevils (Rummel and Summy 1997).

The results of our study clarify long-standing misconceptions regarding the nature of the adult boll weevil dormancy. In addition, newly discovered aspects of the dynamics of diapause termination in the boll weevil contribute to a more comprehensive knowledge of its overwintering ecology. Finally, the information reported here facilitates the construction of a conceptual framework regarding boll weevil diapause and overwintering ecology that may provide focus to future research to the benefit of eradication and management programs in the tropics and subtropics.

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