

# DIET-MEDIATED TERMINATION OF BOLL WEEVIL DORMANCY

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## Abstract

Most studies of diapause in the boll weevil (*Anthonomus grandis* Boheman) have focused on factors influencing its induction. A more complete understanding of this survival mechanism also requires knowledge of the factors controlling diapause termination. Termination responses of diapausing weevils exposed to a known reproductive diet (cotton squares) were evaluated. In separate experiments, the reproductive diet was provided either immediately after a 14-d diapause induction period, or after 1, 3, or 5 weeks of starvation following diapause induction. Male weevils did not terminate diapause when exposed to the reproductive diet immediately after the diapause induction period, while the proportion of diapause in females decreased from 0.92 to 0.67 after the diet switch. When weevils were starved between diapause induction and exposure to the reproductive diet, males terminated diapause more frequently as the duration of starvation increased, while females terminated diapause at comparable rates after all durations of starvation. Females remaining in diapause despite exposure to the reproductive diet more often declined to feed than females terminating the diapause. Feeding responses of males were less consistent and more difficult to interpret. Our results indicate marked differences in the termination responses of the respective weevil sexes, and suggest the potential roles of energetically expensive activities such as flight should be examined for their influence on the propensity of weevils to feed and terminate diapause.

## Introduction

Adequate understanding of the dynamics of diapause in the boll weevil, *Anthonomus grandis* Boheman, is essential for development of optimal management and suppression strategies. Since Brazzel and Newsom (1959) first reported the occurrence of an adult diapause in the boll weevil, the factors inducing diapause have been extensively studied (Earle and Newsom 1964, Lloyd et al. 1967, Spurgeon and Raulston 1998a, Wagner and Villavaso 1999). Much less effort has been devoted to examining termination of this physiological state, and published reports are limited to that of Guerra et al. (1982). Those authors reported that trap-collected diapausing boll weevils began to oviposit, indicating termination of the dormancy, within 2 – 5 d of exposure to squares of cotton (*Gossypium hirsutum* L.). On the basis of these and other observations, Guerra et al. (1982) suggested the boll weevil dormancy was most accurately characterized as a quiescence. However, neither weevil histories nor ages were known in these studies. Further, diapause status of individual weevils was based on observation of fat bodies through the dorsal cuticle. Spurgeon et al. (2003) indicated conditions of the reproductive and alimentary tracts influenced fat body ratings, and recommended that fat bodies be rated only with the dorsal cuticle removed. Thus, re-examination of diet-mediated diapause termination using weevils of known age, history, and physiological condition is warranted. Herein we report preliminary analyses of two experiments to examine termination of boll weevil diapause in response to exposure to cotton squares immediately after the diapause induction period, and after specified periods of starvation.

## Materials and Methods

Adult boll weevils were reared from infested squares picked from plants in commercial cotton fields in Brazos and Burleson Cos., Texas. Squares were held within 20 X 20 X 20-cm screened plexi-glass cages in an environmental chamber maintained at  $29.4 \pm 1^\circ\text{C}$  with a photoperiod of 13:11 [L:D] h. Beginning 5 days after collection, squares were examined daily for the presence of pupae. Pupae were held until eclosion in 100 X 15-mm Petri plates containing a thin layer of moistened vermiculite. Plates of pupae were held under the same environmental conditions as collected squares, and were observed twice daily for newly eclosed adults. Adults, separated by sex using the method of Sappington and Spurgeon (2000), were assigned to experimental treatments on the day of eclosion.

### Termination Response Immediately after Diapause Induction

Diapausing boll weevils were obtained by providing a boll diet similar to that used by Spurgeon (2002). Mixed-sex groups of 50 weevils (25 ♀, 25 ♂) were held in 12.5 X 12.5 X 12.5-cm screened plexi-glass cages. Weevils in each cage were provided water in a 29.5-ml plastic diet cup with a cotton wick extending through the lid, and four bolls (20 – 25 mm diameter) which were replaced three times weekly. Environmental conditions were the same as those used for rearing adults.

After 14 d of boll feeding, 10 weevils of each sex were randomly selected from each cage and dissected. Weevils were classified as diapausing or reproductive using the criteria of Spurgeon et al. (2003). Diapausing weevils contained hypertrophied fat bodies consisting of distinct, well-formed, bright white globules. Diapausing females exhibited no ovary development or contained only pre-vitellogenic oocytes; males exhibited small testes covered with an opaque layer of yellow fat. Weevils

were classified as reproductive if fat bodies were dull-colored and sheet-like, if ovaries contained oocytes with yolk, or if testes were not opaque with fat.

Weevils remaining after dissection were provided a square diet known to promote a high incidence of reproductive development (Spurgeon and Raulston 1998b). Each weevil was individually confined in a 100 X 15-mm Petri plate containing a short section (~1 cm) of water-saturated cotton wick, and was provided a single square (5 – 9 mm diameter, with intact bracteoles) daily. During the termination period squares were examined daily for signs of feeding, which was indicated by punctures that penetrated the floral cup and one or more anther sacs. In addition, squares and Petri plates of females were examined for eggs. Males were provided squares for 14 d, after which they were dissected to determine diapause status. Because oviposition signified the termination of dormancy, females that oviposited were dissected on the day oviposition was detected. Females that did not oviposit during the termination period were dissected and classified after 14 d on the square diet. The experiment was repeated four times.

### **Termination Response after Starvation**

Diapausing weevils were obtained as previously described except that single-sex groups of 75 weevils were provided seven bolls three times weekly in 20 X 20 X 20-cm screened plexi-glass cages. At the end of the 14-d diapause induction period, 20 weevils randomly selected from each cage were dissected to determine diapause status. Food in the cages was replaced by a 12 X 8-in. crumpled craft paper refuge before remaining weevils were returned. During the starvation period weevils were held in an environmental chamber at  $23.9 \pm 1^\circ\text{C}$  with a 13:11 [L:D] h photoperiod. After each of 1, 3, and 5 weeks of starvation, 10 weevils were randomly selected for introduction to the previously described reproductive diet. Experimental procedures during the termination period were identical to those previously described. Weevils remaining after removal of the last termination cohort (5 weeks) were dissected to determine the impact of starvation on diapause status. The experiment was repeated three times.

### **Statistical Analyses**

Data from the experiment examining termination immediately following diapause induction were analyzed by ANOVA using the SAS procedure PROC MIXED (SAS Institute 2001). Data for the two weevil sexes were analyzed separately. For each repetition of the experiment the proportion of weevils classified as diapausing was calculated. The proportion of weevils diapausing at the end of the induction period and after the termination period, respectively, were arcsine-transformed (Zar 1984) and compared. The ANOVA model contained the fixed effect of treatment (at the end of the diapause induction period or after the termination period) and the random effect of replication. Mortality during the diapause induction and termination periods resulted in variable sample sizes for estimates of the proportions of weevils diapausing, so the ANOVA was weighted by the respective sample sizes. Least-squares means reported are un-transformed proportions. The numbers of days during the termination period on which weevils failed to feed were also compared among weevils classified as diapausing or reproductive using PROC MIXED. Separate analyses were conducted for each sex, and the respective ANOVA models contained the fixed effect of diapause classification and the random effect of replication.

Data from the experiment examining the influence of starvation on termination were also analyzed using PROC MIXED (SAS Institute 2001). Because the number of experimental units (cages) available were insufficient to allow a factorial treatment structure, the data represented five feeding treatments including two baselines that were not fed squares (at the end of the diapause induction period and five weeks later), and three termination treatments fed squares (after 1, 3, and 5 weeks of starvation). The proportion of weevils diapausing in each treatment was transformed as previously described and analyzed for each sex by weighted ANOVA. Each ANOVA model contained the fixed effect of feeding treatment, and random effects of replication and the replication by feeding treatment interaction. Means corresponding to the five treatments were separated using the ADJUST=TUKEY option of the LSMEANS statement (SAS Institute 2001). As in the previous experiment, the numbers of days weevils failed to feed were compared among the termination treatments and between diapause classifications for each sex using PROC MIXED. The ANOVA models contained fixed effects of feeding treatment, diapause status, and their interaction, and random effects of replication, the replication by treatment interaction, and the replication by diapause status interaction. Differences among levels of feeding treatment were detected using the LSMEANS statement as previously described.

## **Results and Discussion**

### **Termination Response Immediately after Diapause Induction**

Male weevils provided squares after the 14-d diapause induction period failed to terminate diapause ( $F = 0.03$ ;  $df = 1, 6$ ;  $P = 0.87$ ). The proportion of males classified as diapausing at the end of the induction period was  $0.78 \pm 0.08$  (mean  $\pm$  SE) compared with  $0.78 \pm 0.07$  after the termination period. In contrast, a significantly lower proportion of females were classified as diapausing after the termination period ( $0.67 \pm 0.16$ ) than at the end of the diapause induction period ( $0.92 \pm 0.10$ ;  $F = 8.02$ ;  $df = 1, 6$ ;  $P = 0.03$ ). Thus, the two weevil sexes exhibited markedly different termination responses when exposed to squares immediately after diapause induction.

Weevils classified as reproductive at the end of the termination period had fed more frequently than those classified as diapausing. During the 14-d termination period males that were ultimately classified as diapausing ( $n = 43$ ) declined to feed on an average of  $6.75 \pm 0.76$  d compared with an average of  $3.68 \pm 1.17$  d for males classified as reproductive ( $n = 12$ ;  $F = 6.75$ ;  $df = 1, 53$ ;  $P = 0.01$ ). Females of both classifications tended to feed more frequently than males, but again diapausing females ( $n = 38$ ) declined to feed more often than females classified as reproductive ( $n = 18$ ) (diapausing,  $4.71 \pm 0.47$  d; reproductive,  $1.61 \pm 0.68$  d;  $F = 14.27$ ;  $df = 1, 54$ ;  $P < 0.01$ ). Because no termination response was observed for males, differences among individual males in the propensity to feed apparently reflected corresponding differences in diapause status at the beginning of the termination period, and thus were not a consequence of termination. In contrast, females exhibited a significant termination response that was also associated with an increased propensity to feed. These results do not demonstrate whether extensive feeding by females played an integral role in termination or was simply an indicated of termination. Because females tended to feed (and thus receive host cues) more readily than males, is it possible that differences between sexes in the termination response were related to their respective propensities to feed. This possibility suggests the need for further examination of the role of feeding in the termination process.

### **Termination Response after Starvation**

Duration of the starvation period after diapause induction significantly influenced the termination response of male weevils ( $F = 10.86$ ;  $df = 4, 8.87$ ;  $P < 0.01$ ). The proportion of diapause was similar for weevils dissected immediately after the diapause induction period, those starved for 5 weeks after diapause induction, and those provided the reproductive diet for 14 d beginning 1 week after diapause induction (Table 1). However, the termination response tended to increase with increasing time of starvation  $>1$  week in duration (Table 1). Differences among treatments were also observed for female weevils ( $F = 21.37$ ;  $df = 4, 15$ ;  $P < 0.01$ ), but termination response of weevils fed squares was similar irrespective of duration of the starvation period (Table 1). Also, the proportion of diapause corresponding to females dissected after 5 weeks of starvation was significantly higher than for females dissected immediately after the diapause induction period (Table 1). This difference is best explained by mortality of reproductive females during the starvation period.

In this experiment, the mean number of days males on the reproductive diet failed to feed were similar among starvation periods (1 week,  $5.86 \pm 0.90$  d; 3 weeks,  $6.79 \pm 0.68$  d; 5 weeks,  $6.86 \pm 0.56$  d;  $F = 0.56$ ;  $df = 2, 112$ ;  $P = 0.57$ ) and between weevils classified as diapausing ( $7.18 \pm 0.54$  d) or reproductive ( $5.82 \pm 0.81$  d;  $F = 1.97$ ;  $df = 1, 8.66$ ;  $P = 0.19$ ). The mean number of days females on the reproductive diet did not feed were also similar among starvation periods (1 week,  $3.43 \pm 0.48$  d; 3 weeks,  $3.72 \pm 0.52$  d; 5 weeks,  $4.86 \pm 0.45$  d;  $F = 2.69$ ;  $df = 2, 116$ ;  $P = 0.07$ ), but differences were detected between females that were diapausing ( $5.81 \pm 0.36$  d) or reproductive ( $2.20 \pm 0.42$  d) at the end of the termination period ( $F = 42.13$ ;  $df = 1, 116$ ;  $P < 0.01$ ). These results suggest the role of feeding in diapause termination is more prominent in females than in male weevils. However, interpretation of these results is not straightforward for several reasons. First, our data indicate only whether feeding occurred and do not provide estimates of the extent of feeding. It is obvious that some weevils that fed did not terminate diapause. Provided the host cues eliciting termination are received primarily through feeding, it is possible some weevils that fed did so at rates too low to receive cues sufficient to cause termination. Secondly, egg production is energetically demanding, and thus termination may result in a more consistent feeding response in females than in males, especially when fat reserves are already substantial. Finally, males exhibiting the diapause characters are capable of mating (Spurgeon and Raulston 1998a), and the consequences of maintaining the diapause characters in the presence of a reproductive host are different for males than for females. These results suggest the possibility that one or more additional behaviors that induce feeding, through the expenditure of energy or by acting as a behavioral primer, may be necessary for a more immediate termination response from males. In that case, additional activities such as flight should be examined for a possible role in the termination process.

Our results indicate markedly different diapause termination responses of male and female boll weevils exposed to a reproductive diet. At least part of this difference may be related to the reception of host cues because females exhibited a greater apparent propensity to feed on the reproductive diet. Although we elicited statistically significant termination responses from both weevil sexes these responses were more limited than anticipated. In that respect our results differ substantially from those of Guerra et al. (1982), who observed a more rapid and complete termination response. Differences in our respective results may have been caused by differences in the ages, histories, and physiological states of the weevils studied. It should also be considered that the reproductive diet we employed during the termination period is not an optimal diet for reproductive development (Spurgeon and Raulston 1998b), but rather represents a diet that is practical for use in experiments involving relatively large numbers of weevils. We anticipate that exposure of diapausing weevils to a more ample supply of squares or to intact fruiting plants may evoke a more pronounced termination response. In light of these hypotheses, potential roles of energetically expensive activities such as flight should be examined for their influence on the propensity of weevils to feed and thus terminate diapause.

### **Disclaimer**

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Table 1. Diapause status of boll weevil adults provided a diapause inducing (boll) diet for 14 d and then switched or not switched to a reproductive (square) diet for 14 d after specified starvation periods.

Weeks after Diapause Induction	Switched to Reproductive Diet	Least-Squares Mean ( $\pm$ SE) Proportion Diapausing <sup>a</sup>	
		Male	Female
0	N	0.91 $\pm$ 0.04 ab	0.89 $\pm$ 0.04 b
1	Y	0.89 $\pm$ 0.05 abc	0.55 $\pm$ 0.05 c
3	Y	0.75 $\pm$ 0.05 bc	0.65 $\pm$ 0.06 c
5	Y	0.63 $\pm$ 0.04 c	0.51 $\pm$ 0.05 c
5	N	1.00 $\pm$ 0.04 a	0.98 $\pm$ 0.05 a

<sup>a</sup>Least-squares means followed by the same letter are not significantly different ( $\alpha = 0.05$ ; Tukey-Kramer test).