# BOLL WEEVIL REPRODUCTIVE DEVELOPMENT UNDER SELECTED FEEDING REGIMES

**D. W. Spurgeon and J. R. Raulston**
**USDA, ARS**
**Subtropical Agricultural Research Laboratory**
**Weslaco, TX**

## Abstract

Impacts of a measure of square quality (oviposition punctured or uninfested) and frequency of food replacement (daily or on alternating days) on reproductive development of adult boll weevils were investigated in a preliminary study. Boll weevils of each sex were supplied either a freshly collected, oviposition punctured or uninfested square, either daily or on alternate days under controlled environmental conditions. Males were relatively insensitive to feeding regime and no differences in reproductive parameters or fat body condition could be detected among groups. Reduced square quality or frequency of food replacement reduced the numbers of eggs and mature eggs produced, with frequency of food replacement having the most influence. These factors also combined to reduce the proportion of females producing mature eggs by 6 d of age. Mid gut examinations of 4-d-old weevils of both sexes indicated that occurrence of substantial feeding was reduced when squares were supplied on alternate days compared with a regime of daily square replacement. These data indicate that reproductive development of female boll weevils is subtly influenced by feeding regime. However, these relatively minor influences could introduce sizable errors into boll weevil reproductive biology experiments when combined with other suboptimal conditions.

## Introduction

Recent investigations at this laboratory, regarding boll weevil reproductive biology, diapause, and overwintering survival, have resulted in identification of a number of potentially influential experimental artifacts associated with such studies. In preliminary experiments, Spurgeon and Raulston (1996) found that crowding of weevils combined with characteristics of food squares (oviposition punctured or uninfested) markedly influenced reproductive commitment of female boll weevils. High levels of crowding and poor host quality caused females to assume the morphological characteristics of diapause. Subsequent moderation of these conditions increased reproductive commitment and decreased the apparent frequency of diapause. Their experimental design did not separate the effects of crowding, host quality, and food availability. To circumvent such artifactual effects in our research we have established a standard feeding regime of a single unpunctured square each day per boll weevil confined individually. However, the availability of adequate numbers of unpunctured, pesticide-free squares is often limiting. Also, considerable labor savings could be achieved if weevils could be fed on alternating days or punctured squares could be fed without impacting reproductive development. Thus, we performed a preliminary investigation of the impacts of square quality (oviposition punctured or uninfested) and frequency of food replacement (daily or on alternating days) on reproductive development of adult boll weevils.

## Materials and Methods

Adult boll weevils of known age were obtained by collecting pupae from field-collected infested squares. Infested squares that had not abscised were collected and held in screen cages within environmental cabinets at 29.4°C and with a 13:11 h [L:D] photoperiod. Pupae were harvested when subsamples of the squares indicated that ≥75% of the larvae had pupated. Pupae were held under the same conditions in petri plates containing a shallow layer of moistened vermiculite and checked for adult eclosion twice daily. Upon eclosion, callow adults were sexed and assigned to treatment regimes. Adult weevils for the experiment were collected over a 2-d period.

Tested feeding regimes included: 1) a single uninfested square supplied daily, 2) a single oviposition punctured square supplied daily, 3) a single uninfested square supplied on alternating days, and 4) a single oviposition punctured square supplied on alternating days. Food squares were collected from the field, rinsed with distilled water, and either supplied immediately or stored in a refrigerator for ≤2 d. Between 35 and 40 weevils of each sex were assigned to each treatment regime. All weevils were held individually in petri plates containing the food square and a short section of dental wick saturated with distilled water. Adults were held in an environmental chamber at 29.4°C. Photoperiod was initially set at 13:11 h [L:D], but malfunction of a timer resulted in a photoperiod of about 11.5:12.5 h [L:D] for the first four days of the experiment.

Ten weevils of each sex from each feeding regime were dissected at 2, 4, and 6 d of age. Assessments of reproductive and fat body condition relied heavily on descriptions of Burke (1959) and Brazzel and Newsom (1959), combined with our own observations. Weevils were classified as fat if the fat body obscured most of the internal organs, intermediate if the fat body was well developed but portions of the digestive tract or reproductive organs were visible, and lean if internal organs were generally visible, regardless of the presence of fat. Presence and numbers of eggs and mature eggs were recorded. Mature eggs were recorded as present if the female had oviposited, but oviposited eggs were not counted. Testes were recorded as small if the long axis length was <½ the combined length of the meta- and mesothorax and the abdomen. We previously

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:982-984 (1997)
National Cotton Council, Memphis TN

observed testicular development to follow a relatively distinct and predictable pattern and male testes were rated according to these observations. Testes development was assessed as prereproductive (testes translucent), begining reproductive (opaque center, translucent lobes), or later reproductive (opaque center, cloudy lobes). No atrophied testes were observed. Seminal vesicles were rated according to their sperm content: transparent (no sperm), translucent (sperm present), opaque but small (filled with sperm but not distended), and opaque but large (distended with sperm, prominently club-shaped). In addition to reproductive observations we assessed mid gut condition to aid in interpretation of feeding regime effects. Mid guts were rated as full if >½ of the volume was filled with solid food, intermediate if solid food filled <½ of the volume, trace if masses of solid food were not observed but contents of the gut were colored, and empty if the gut was translucent. Previous observations indicated that mid gut ratings of full or intermediate were typical of actively feeding weevils.

Numbers of eggs and mature eggs were compared among feeding regimes by analysis of variance using the SAS procedure PROC GLM (SAS Institute 1988). Frequencies of reproductive, fat body, and mid gut ratings were compared among feeding regimes in contingency tables using Fisher's Exact Test (EXACT option of the SAS procedure PROC FREQ, SAS Institute 1988).

## Results

No differences in fat body ratings were detected among the feeding regimes ($0.231 \leq P \leq 1.000$). Most weevils were rated as lean (females: 87.5-95%; males 85-92.5%), and a maximum of 2.5% of weevils were rated as fat regardless of sex, feeding regime, or age.

Most weevils had fed actively by 2 d of age, but frequency of substantial feeding decreased with increasing age (Fig. 1). Frequency of feeding was similar among feeding regimes except when weevils were 4 d old. The proportion of both sexes of 4-d-old weevils that fed substantially was reduced when squares were supplied on alternate days (females, $P$=0.018; males, $P$=0.014; Fig. 2). Differences in the frequency of substantial feeding may have been caused by deterioration of the squares supplied on alternate days as these squares appeared drier than those supplied daily at the time of food replacement.

No differences in male reproductive parameters were observed among feeding regimes. Testes condition was classified as later reproductive for 35, 95, and 97.5% of weevils at ages of 2, 4, and 6 d, respectively. Testes size was classified as normal for 70-82.5% of males at the different ages. Seminal vesicles of 87.5% of 2-d-old males contained sperm, and 47.5% of these were opaque but small. At 4 d of age 82.5% of weevils had seminal vesicles distended with sperm, and all dissected males contained seminal vesicles distended with sperm by 6 d of age.

No differences in the proportions of females with eggs were detected among feeding regimes ($0.083 \leq P \leq 0.891$). The proportion of females with eggs increased from 72.5% at 2 d to 90% at 6 d of age (Fig. 3). The proportion of females with mature eggs increased greatly between 2 and 4 d of age (Fig. 3), and no differences among feeding regimes in the proportions of females with mature eggs were detected at 2 ($P$=0.231) or 4 d of age ($P$=0.233). However, feeding regime did influence the proportion of females with mature eggs at 6 d of age ($P$=0.048, Fig. 4). A comparison between frequencies of food replacement indicated that most of the observed differences among feeding regimes could be attributed to this factor ($P$=0.044). Analyses of variance indicated that numbers of both total eggs ($F$=3.20; df=3, 108; $P$=0.026) and mature eggs ($F$=12.87; df=3, 108; $P$<0.001) were influenced by feeding regime (Figs. 5 and 6, respectively). Effects of frequency of food replacement appeared slightly more influential than did square quality in these observations.

## Discussion

Our results indicate that reproductive development and commitment of male boll weevils were relatively insensitive to square quality and frequency of food replacement parameters we examined, although feeding of both male and females weevils were measurably impacted by feeding regime. Reproductive parameters of females were subtly but measurably responsive to feeding regimes. In themselves, the modifications to our standard feeding regime that we examined may not represent serious aritifacts in reproductive biology or diapause studies, but these factors may be manifested more distinctly when combined with other experimental conditions that are suboptimal for reproductive development.

## References

Burke, H. R. 1959. Morphology of the reproductive systems of the cotton boll weevil (Coleoptera, Curculionidae). Ann. Entomol. Soc. Am. 52: 287-294.

Brazzel, J. R. and L. D. Newsom. 1959. Diapause in *Anthonomus grandis* Boh. J. Econ. Entomol. 52: 603-611.

SAS Institute. 1988. SAS user's guide: statistics, version 6.03 ed. SAS Institute, Cary, NC.

Spurgeon, D. W. and J. R. Raulston. 1996. Boll weevil reproductive development responses to crowding and variations in host quality. Proceedings Beltwide Cotton Conferences. 983-987.

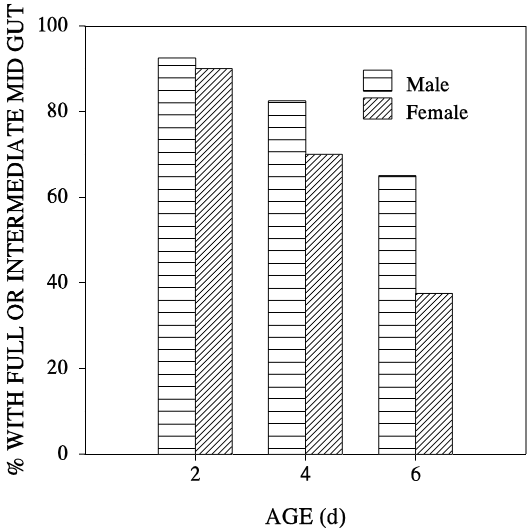


Fig. 1. Proportions of boll weevils at different ages with full or intermediate mid guts indicating substantial feeding.

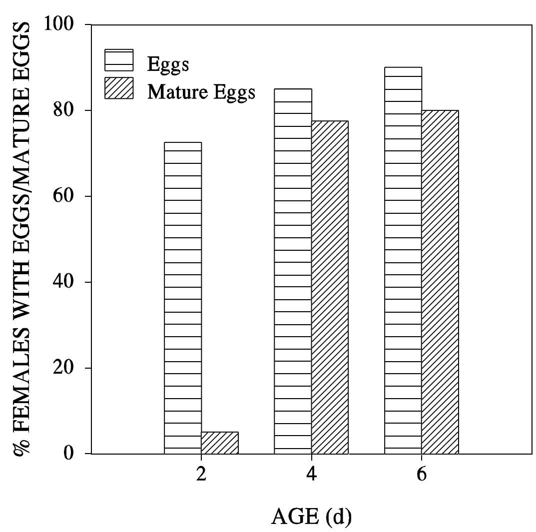


Fig. 3. Proportions of female boll weevils with eggs and mature eggs at different ages.

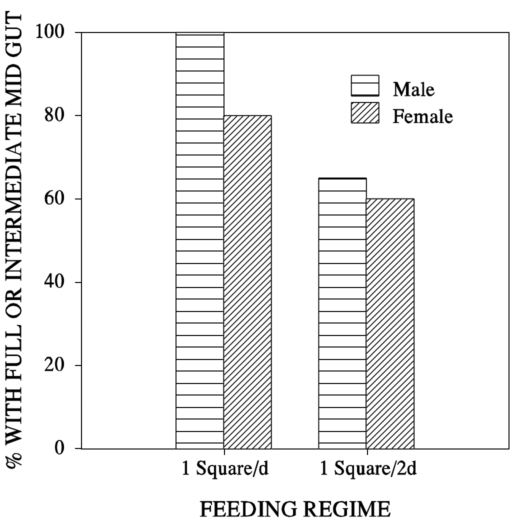


Fig. 2. Proportions of 4-d-old boll weevils under different food replacement regimes with full or intermediate mid guts indicating substantial feeding.

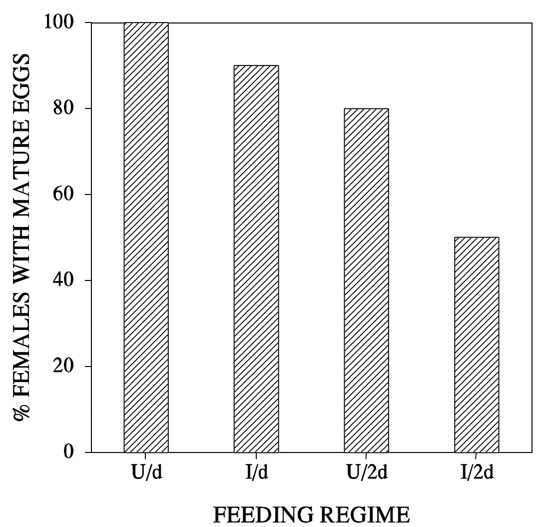


Fig. 4. Proportions of 6-d-old female boll weevils with mature eggs under different feeding regimes (U/d, uninfested square per day; I/d, oviposition punctured square per day; U/2d, uninfested square on alternate days; I/2d, oviposition punctured square on alternate days).

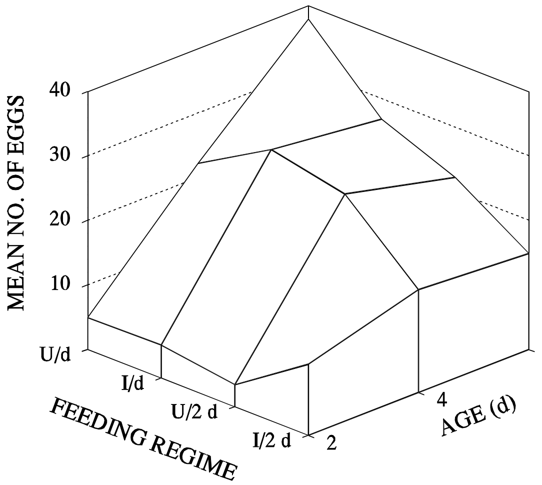


Fig. 5. Mean numbers of eggs per female boll weevil at different ages and under various feeding regimes (U/d, uninfested square per day; I/d, oviposition punctured square per day; U/2d, uninfested square on alternate days; I/2d, oviposition punctured square on alternate days).

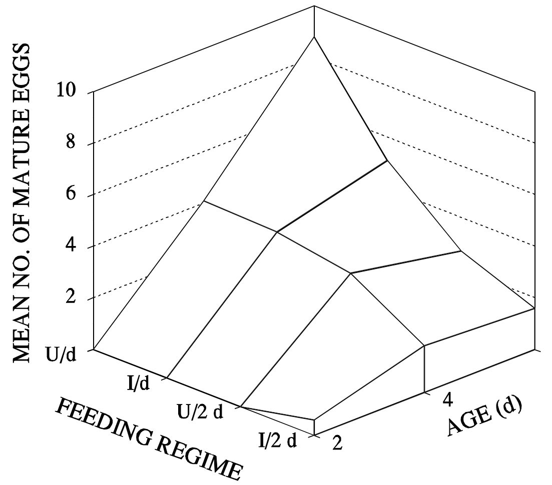


Fig. 6. Mean numbers of mature eggs per female boll weevil at different ages and under various feeding regimes (U/d, uninfested square per day; I/d, oviposition punctured square per day; U/2d, uninfested square on alternate days; I/2d, oviposition punctured square on alternate days).