

# BOLL WEEVIL REPRODUCTIVE DEVELOPMENT UNDER SELECTED TEMPERATURE REGIMES

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## Abstract

Temperature dependence of boll weevil reproductive development was examined at constant temperatures of 12.8, 18.3, 23.9, and 29.4°C and a 13:11 h [L:D] photoperiod by dissecting weevils at specified ages. Logistic curves were fitted to age-frequencies of the presence of eggs, eggs with yolk, mature eggs, early reproductive testes (beginning of visible sperm accumulation in the centers of testis lobes), later reproductive testes (visible sperm accumulation in outer areas of testicular follicles), and filled seminal vesicles. Considerable variation was observed in reproductive development within temperature regimes, but logistic curves generally fit the data well ( $r^2 > 0.94$ ) except at 12.8°C ( $0.652 \leq r^2 \leq 0.950$ ). We observed a marked temperature dependence of boll weevil reproductive development. Exposure to lower temperatures did not induce morphological characteristics of diapause. These results suggest that results of earlier studies of diapause induction should be reexamined because of previous failures to account for differences in physiological ages of weevils among experimental treatments.

## Introduction

Since Brazzel and Newsom (1959) reported the occurrence of a reproductive diapause in the boll weevil considerable effort has been devoted to examining the environmental factors involved in diapause induction. Most studies examining effects of photoperiod and temperature on induction of diapause in adult weevils have involved assessment of diapause status by dissection of weevils after a specified feeding period. The length of this feeding period was typically the same for different induction regimes regardless of differences in temperatures among the regimes (e.g., Earle and Newsom 1964, Lloyd et al. 1967, Tingle and Lloyd 1969, and Wagner and Villavaso 1996).

Because the boll weevil is a poikilotherm, differences in reproductive development among induction regimes could represent an important source of experimental error when diapause is assessed among the various regimes at a fixed chronological age. We examined the temperature dependence of boll weevil reproductive development to assess the potential for such errors.

## Materials and Methods

Adult boll weevils of known age were reared from field-collected infested squares. Infested squares were collected from plants and held in screen cages within environmental cabinets at 29.4°C and a 13:11 h [L:D] photoperiod. Pupae were harvested when subsamples of the squares indicated that  $\geq 75\%$  of the larvae had pupated. Pupae were held in the same cabinets in petri dishes containing a shallow layer of moistened vermiculite. Newly eclosed adults were collected twice daily, sexed, and assigned to temperature treatments. Reproductive development was examined at four temperatures, 12.8, 18.3, 23.9, and 29.4°C. Because Spurgeon and Raulston (1996) reported that crowding and characteristics of food squares could influence reproductive commitment of female weevils, weevils of each sex were confined individually in petri dishes each containing a freshly collected unpunctured square and a short section of dental wick saturated with distilled water. Squares were replaced daily.

Weevils were dissected at fixed intervals based on the results of preliminary experiments. At 12.8°C, 10 weevils of each sex were dissected at three-day intervals from 6 - 36 d after eclosion. At 18.3, 23.9, and 29.4°C weevils were dissected daily at ages of 3 - 18, 2 - 11, and 1 - 8 d, respectively. Fat body and mid gut conditions, presence of juvenile fat, and degree of development of reproductive organs, were recorded. The experiment was repeated once at each temperature.

Assessments of reproductive and fat body condition were based on descriptions of Burke (1959) and Brazzel and Newsom (1959), combined with our own observations. Weevils were considered 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. Because fat body often adhered to the ovaries, presence of eggs, eggs with yolk, and mature eggs was determined by microscopic inspection of the ovaries after they were removed from the abdomen and cleaned of fat tissue. We previously observed testicular development to follow a relatively distinct and predictable pattern and male testes were rated according to these observations. Testis development was assessed as prereproductive (testis translucent, no visible accumulation of sperm), early reproductive (opaque center and translucent lobes, beginning of visible sperm accumulation in the center of the testis), or later reproductive (opaque center and cloudy lobes, visible accumulation in outer areas of testicular follicles). Seminal vesicles were rated according to their sperm content: prereproductive (transparent, no sperm or translucent, sperm present but inconspicuous) or reproductive (seminal vesicles filled with sperm). Laboratory observations have indicated that a seminal vesicle rating of reproductive coincides with the ability of the male weevil to successfully mate.

In addition to reproductive observations we assessed mid gut condition to aid in data interpretation. Mid guts were rated as full if  $>1/2$  of the volume was filled with solid food, intermediate if solid food filled  $<1/2$  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.

Logistic functions were fitted to age-frequencies of the presence of eggs, eggs with yolk, mature eggs, early reproductive testes, later reproductive testes, and filled seminal vesicles using the SAS procedure PROC NLIN (SAS Institute 1988). The form of the model was:  $Y = [e^{(B_0 + B_1 * AGE)} / (1 + e^{(B_0 + B_1 * AGE)})]$  where  $Y$  is the respective reproductive parameter, AGE is the number of days since adult eclosion, and  $B_0$  and  $B_1$  are estimated.

### **Results and Discussion**

Adult weevils frequently contained conspicuous amounts of fat at adult eclosion. This fat was slightly gray and of a finer texture than is typical of adult fat body. Of the 1600 dissected weevils, 1.5% contained enough juvenile fat to be rated as fat. The juvenile fat diminished with increasing age, becoming absent in 15-20 days at 12.8°, 5-7 days at 18.3°, 3-4 days at 23.9°, and 1-2 days at 29.4°C. This pattern did not appear to differ between sexes. Only 1.6% of older weevils dissected were classified as being fat. Most weevils (females, 71.4%; males 75.9%) were rated as lean. Although nearly 25% of the weevils attained a fat body rating of intermediate, no pattern in occurrence of this rating was observed as a function of temperature.

Examinations of mid guts indicated that most weevils at 12.8°C had not fed substantially by 6 d after eclosion. In the second repetition at that temperature, 60-90% of the weevils did not feed actively until 15 d after eclosion. Most weevils had fed by 4 d at 18.3°C, 3 d at 23.9°C, and 2 d after eclosion at 29.4°C.

The logistic functions indicated a strong temperature dependence of female reproductive development. The functions fit to the frequencies of egg presence at different ages (Fig. 1) predicted mean times to presence of eggs of 25.70, 5.53, 2.76, and 1.87 d (12.8°C,  $B_0 = -4.601$ ,  $B_1 = 0.179$ ,  $r^2 = 0.886$ ; 18.3°C,  $B_0 = -5.631$ ,  $B_1 = 1.019$ ,  $r^2 = 0.978$ ; 23.9°C,  $B_0 = -12.221$ ,  $B_1 = 4.421$ ,  $r^2 = 0.981$ ; 29.4°C,  $B_0 = -24.940$ ,  $B_1 = 13.337$ ,  $r^2 = 0.985$ , respectively). Functions fit to frequencies of presence of eggs with yolk (Fig. 2) predicted mean times to presence of eggs with yolk of 37.85, 7.24, 4.39, and 2.11 d (12.8°C,  $B_0 = -6.246$ ,  $B_1 = 0.165$ ,  $r^2 = 0.652$ ; 18.3°C,  $B_0 = -6.025$ ,  $B_1 = 0.832$ ,  $r^2 = 0.941$ ; 23.9°C,  $B_0 = -10.037$ ,  $B_1 = 2.284$ ,  $r^2 = 0.986$ ; 29.4°C,  $B_0 = -5.363$ ,  $B_1 = 2.542$ ,  $r^2 = 0.977$ , respectively). The predicted mean time to presence of eggs with yolk at 12.8°C may not be reliable because this prediction is beyond the range of our observations. Mean time to presence of mature

eggs could not be predicted at 12.8°C because only 3 females with mature eggs were ever observed. Mean predicted times to presence of mature eggs at the other temperatures were 9.97, 5.40, and 3.06 d (18.3°C,  $B_0 = -4.844$ ,  $B_1 = 0.486$ ,  $r^2 = 0.928$ ; 23.9°C,  $B_0 = -11.839$ ,  $B_1 = 2.191$ ,  $r^2 = 0.984$ ; 29.4°C,  $B_0 = -5.998$ ,  $B_1 = 1.957$ ,  $r^2 = 0.965$ , respectively) (Fig 3).

Logistic functions fit to frequencies of occurrence of early (Fig. 4) and late reproductive testes (Fig. 5), and filled seminal vesicles (Fig. 6) also indicated a strong temperature dependence of male reproductive development. Mean times to early reproductive testes of 22.20, 6.11, 3.14, and 1.95 d (12.8°C,  $B_0 = -6.615$ ,  $B_1 = 0.298$ ,  $r^2 = 0.950$ ; 18.3°C,  $B_0 = -11.804$ ,  $B_1 = 1.932$ ,  $r^2 = 0.989$ ; 23.9°C,  $B_0 = -7.282$ ,  $B_1 = 2.318$ ,  $r^2 = 0.989$ ; 29.4°C,  $B_0 = -56.273$ ,  $B_1 = 28.830$ ,  $r^2 = 0.998$ , respectively) were predicted. Predicted mean times to presence of late reproductive testes were 34.72, 10.94, 6.15, and 4.09 d (12.8°C,  $B_0 = -34.334$ ,  $B_1 = 0.989$ ,  $r^2 = 0.872$ ; 18.3°C,  $B_0 = -13.811$ ,  $B_1 = 1.263$ ,  $r^2 = 0.975$ ; 23.9°C,  $B_0 = -8.728$ ,  $B_1 = 1.420$ ,  $r^2 = 0.976$ ; 29.4°C,  $B_0 = -5.036$ ,  $B_1 = 1.230$ ,  $r^2 = 0.974$ , respectively), while predicted mean times to filled seminal vesicles were 32.5, 7.89, 4.63, and 2.17 d (12.8°C,  $B_0 = -9.425$ ,  $B_1 = 0.290$ ,  $r^2 = 0.916$ ; 18.3°C,  $B_0 = -8.578$ ,  $B_1 = 1.087$ ,  $r^2 = 0.982$ ; 23.9°C,  $B_0 = -10.488$ ,  $B_1 = 2.267$ ,  $r^2 = 0.995$ ; 29.4°C,  $B_0 = -7.830$ ,  $B_1 = 3.602$ ,  $r^2 = 0.998$ , respectively). Laboratory observations have indicated that the stage of male reproductive development to which we refer as filled seminal vesicles coincides with the ability of the male to successfully mate. The temperature dependence of male reproductive development is such that the ability to mate is attained at approximately the same time that females first contain eggs with yolk, and is closely followed by the presence of mature eggs. Thus, our data indicate that the temperature dependence of physiological attainment of sexual maturity of males and females is similar. The extended development times observed for both sexes at 12.8°C indicate that this temperature is close to the lower developmental threshold.

The proportion of weevils committing to reproduction was very high in our study, although our data probably underestimated the actual degree of reproductive commitment. Eleven weevils (10 females, 1 male) of ages at which evidence of reproductive development could reasonably be expected failed to show such development. Eight of these weevils (7 females, 1 male) contained no evidence of fat body or recent feeding, and were characterized by shrunken abdomens or very soft cuticle. Although mortality among the temperature regimes was  $<5\%$ , these weevils may have failed to commit to reproduction because they were infirm. Only three of the 1600 weevils dissected (18.3°C, one female; 29.4°C, two females) displayed the morphological characteristics of diapause. Thus, diapause was not induced by low temperature conditions in our study.

A small number of dissected males (14, 1.75%) contained testes that were similar to the atrophied testes described by Brazzel and Newsom (1959). This condition occurred only in the physiologically oldest weevils. Weevils attaining this condition in our study had previously committed to reproduction and possessed seminal vesicles distended with sperm. Observations made during the serial dissections indicated that development of this condition began with deposition of a thin layer of yellow fat tissue on the surface of later reproductive testes. The layer of fat subsequently became more opaque because of continued deposition, concentration caused by decrease in testis size, or both. Brazzel and Newsom (1959) reported that atrophied testes were filled with fat globules. Our observations were different. We found that removal of external fat resulted in a relatively translucent testis, and no internal fat was detected. Despite careful cleaning, enough surface fat remained to prevent accurate determination of sperm presence in crushed testes. The ability of such males to successfully inseminate females was previously demonstrated (Spurgeon and Raulston 1996), and observations in our laboratory have indicated that these males are capable of replenishing the sperm supply in the seminal vesicles. Moreover, we have observed that a male in this condition, confined with multiple females, may maintain the quantity of sperm in the seminal vesicles at a low level because of continued mating activity. Immediately after mating the seminal vesicles may be empty, appearing to fit exactly Brazzel and Newsom's (1959) description for weevils in firm diapause. In the present study, males were not allowed access to mates, and atrophy of the testes may represent a mechanism of regulating sperm production.

Our data demonstrate a marked temperature dependence of boll weevil reproductive development sufficient to cause considerable error in investigations of diapause induction when dissections are performed at fixed chronological rather than physiological ages. These potential errors may become more serious when combined with other factors known to influence reproductive commitment, such as feeding regime or food quality. Our results suggest that reexamination of earlier studies of diapause induction, in which differences in physiological ages of weevils among experimental treatments were not considered, may be warranted.

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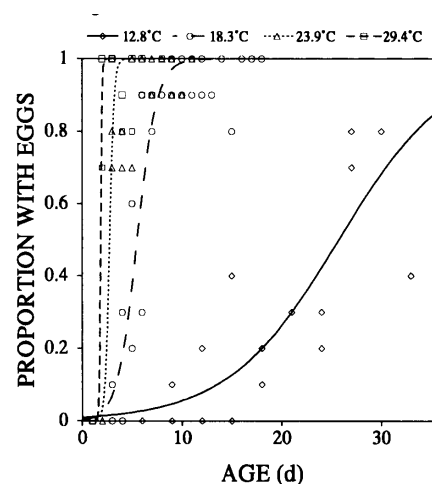
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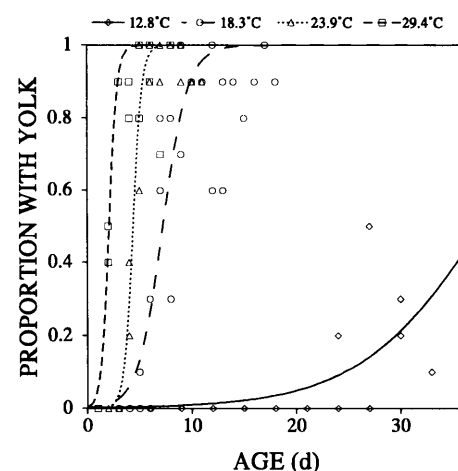
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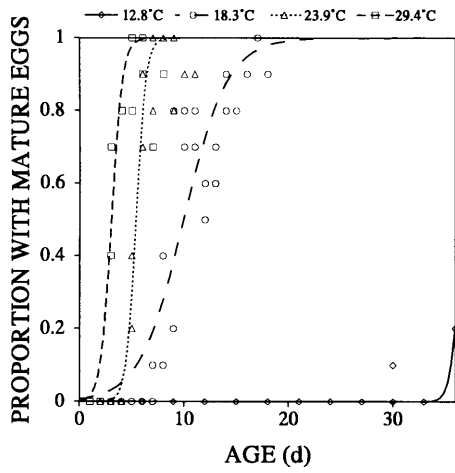
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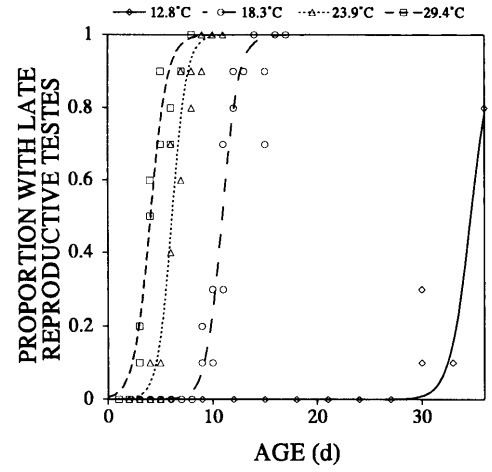
**Figure 1** Observed and predicted (logistic equation) relationships between age and presence of eggs in female boll weevils held under constant temperatures.



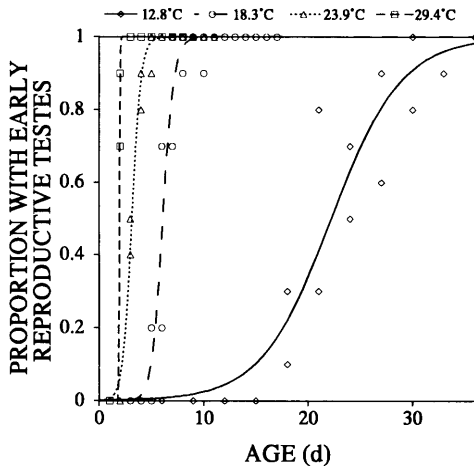
**Figure 2** Observed and predicted (logistic equation) relationships between age and presence of eggs with yolk in female boll weevils held under constant temperatures.



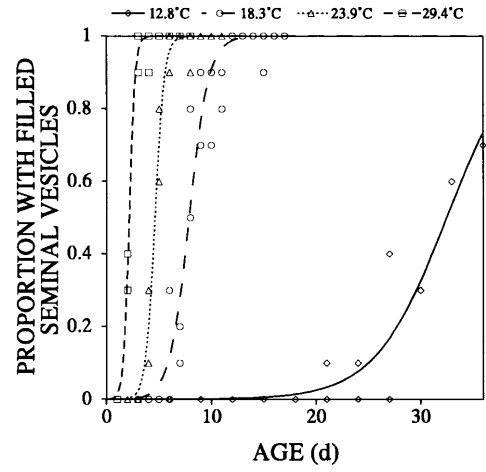
**Figure 3** Observed and predicted (logistic equation) relationships between age and presence of mature eggs in female boll weevils held under constant temperatures.



**Figure 5** Observed and predicted (logistic equation) relationships between age and occurrence of late reproductive stage testes in male boll weevils held under constant temperatures.



**Figure 4** Observed and predicted (logistic equation) relationships between age and occurrence of early reproductive stage testes in male boll weevils held under constant temperatures.



**Figure 6** Observed and predicted (logistic equation) relationships between age and presence of filled seminal vesicles in male boll weevils held under constant temperatures.